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A new species of *Thamnophis* (Serpentes, Colubridae) from Jalisco, Mexico, with a discussion on the phylogeny, taxonomy, and distribution of snakes related to *Thamnophis scalaris*

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Abstract

Garter snakes in the genus *Thamnophis* from Mexico have a long and convoluted taxonomic history. From 2015 to 2022, we conducted a comprehensive sampling of Mexican *Thamnophis* species, aiming to link molecular phylogenies with the recognized species related to *T. scalaris* in the highlands of Mexico. Here, we present an analysis of mitochondrial DNA to resolve the status of two enigmatic highland *Thamnophis* populations. Our research resulted in the identification and morphological characterization of a previously undescribed *Thamnophis* species from the state of Jalisco in western Mexico. We also clarify the identity and relationships of several previously enigmatic populations of *Thamnophis*. This work presents new data for *Thamnophis* phylogenetics from the Mexican highlands and offers a framework for future conservation efforts.

Resumen

Las culebras del género *Thamnophis* de México tienen una historia taxonómica larga y complicada. De 2015 a 2022, realizamos un muestreo integral de las especies de *Thamnophis* de México, con el objetivo de conciliar la filogenética molecular con las delimitaciones de especies establecidas en el complejo relacionado con *T. scalaris* en las tierras altas de México. Aquí presentamos un análisis del ADN mitocondrial para resolver las trayectorias evolutivas y el estado de dos enigmáticas poblaciones de *Thamnophis* de las tierras altas. Nuestra investigación resultó en la identificación y caracterización morfológica de una especie de *Thamnophis* no descrita previamente del estado de Jalisco en el oeste de México. También aclaramos la identidad y las relaciones de varias poblaciones de *Thamnophis* previamente enigmáticas. Este trabajo presenta nuevos datos para nuestra comprensión de la filogenética de *Thamnophis* del altiplano mexicano y ofrece un marco para futuros esfuerzos de conservación.

Kurzfassung

Strumpfbandnattern der Gattung *Thamnophis* aus Mexiko haben eine lange und verworrene taxonomische Geschichte. Von 2015 bis 2022 führten wir eine umfassende Probenahme der *Thamnophis*-Arten Mexikos durch, mit dem Ziel die Phylogenetik mit etablierten Artenabgrenzungen im *T. scalaris* Komplex im Hochland Mexikos in Einklang zu bringen. Hier präsentieren wir eine Analyse der mitochondrialen DNA, um die Evolutionsverläufe und den Status zweier rätselhafter *Thamnophis*-Populationen aufzuklären. Unsere Forschung führte zur Identifizierung und Charakterisierung einer bisher unbeschriebenen *Thamnophis*-Art aus dem Bundes-

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staat Jalisco im Westen Mexikos. Wir klären auch die Identität und Beziehungen mehrerer bisher rätselhafter *Thamnophis*-Populationen. Diese Arbeit stellt neue Daten für unser Verständnis der Phylogenetik von *Thamnophis* aus dem mexikanischen Hochland vor und bietet einen Rahmen für zukünftige Schutzbemühungen.

Key Words

Adelophis, conservation, *errans*, *godmani*, pine-oak woodland, Mexican Transverse Ranges, *scaliger*

Palabras clave

Adelophis, bosque pino-encino, conservación, Eje Neovólcanico, *errans*, *godmani*, *scaliger*

Schlüsselwörter

Adelophis, Eje Neovólcanico, *errans*, *godmani*, Kiefern-Eichen Wald, Naturschutz, *scaliger*

Introduction

The taxonomy of the Longtail Alpine Garter Snake, *Thamnophis scalaris* Cope, 1861, has been a subject of confusion since its description. Historically, *Thamnophis scalaris* was confused with both *Thamnophis scaliger* (Jan, 1863) and *Thamnophis godmani* (Günther, 1894), and these two species were considered subspecies of *T. scalaris* at one time (Smith 1942). The type specimen of *T. scalaris* was collected near Xalapa, Veracruz (Cope, 1861). Later, Cope (1887) referred additional specimens from Orizaba, Veracruz, and nearby localities in Puebla to *T. scalaris*. Jan (1863) described *T. scaliger* but did not specify a type locality, leaving the association of this name with certain *Thamnophis* populations unclear. However, it should be noted that Jan had access to material collected by François Sumichrast in Xalapa and other areas of Veracruz at the time he described *T. scaliger* (Grünwald et al. 2015, p. 402).

The first accounts of *Thamnophis scalaris* from Jalisco were derived from material collected by A. C. Buller in 1892, which Boulenger (1893) reported as "*Tropidonotus scalaris*." His concept of *T. scalaris* was based on seven specimens from Jalisco (which we now recognize in this study as a distinct species) and one specimen from Oaxaca, which was likely mislabeled. Later, Smith (1942) reviewed the *Thamnophis* of Mexico and considered *T. scaliger* and *T. godmani* to be subspecies of *T. scalaris*. Smith et al. (1950) also examined specimens of *Thamnophis* at the Natural History Museum, London, and categorized the Jalisco specimens reported by Boulenger (1893) within their concept of *T. scalaris scaliger*. Subsequently, Rossman and Lara-Gongora (1997) removed *Thamnophis scaliger* from the synonymy of *T. scalaris* and proposed a neotype for *T. scaliger* from Mexico City. This was done in order to preserve the name "*T. scaliger*" for the population that Smith (1943) and Smith et al. (1950), among others, had associated with it. Jan's (1863) description was extremely vague, and Smith (1942) recognized this and ignored the issue regarding the lost "*T. scaliger*" type and decided to apply the name to one of the morphologically distinct populations that he had at hand. At the time Rossman and Lara-Gongora (1997) were investigating the group, most authors were following Smith's (1942) concept of *T. scaliger*, so Rossman and Lara-Gongora (1997) wanted to stabilize the name *T. scaliger*, and thus they neotypified the name for populations in central Mexico.

Rossman and Lara (1997) helped clarify the relationships between *T. scalaris* and *T. scaliger*, and they considered *T. scalaris* to be distributed along the Mexican Transverse Ranges from western Jalisco east to central Veracruz and eastern Puebla. These authors partially recognized the distinct nature of the Jalisco *T.* "*scalaris*" from typical *T. scalaris* in their review and grouped these individuals as part of their "western *T. scalaris*." However, they continued to confuse these populations with true "*T. scalaris*" from eastern Michoacán and the high mountains around Mexico City. Unfortunately, these authors did not publish more specific data that would facilitate comparisons between the different geographical populations of *T. scalaris*.

Against this historical backdrop, our research was motivated by the discovery of garter snakes in Jalisco that were morphologically distinct and could not be assigned to any known species of *Thamnophis*. To resolve the taxonomic status of these populations, we undertook a comprehensive molecular and morphological analysis of Mexican highland *Thamnophis*. Morphologically, the Jalisco population appeared to be intermediate between *T. scalaris* and *Thamnophis errans* Smith, 1942, and molecular data were needed to determine their specific identity. This study presents these molecular findings and provides a morphological description of the Jalisco population, which we recognize as a new species. In addition, we resolve some longstand-

ing taxonomic conundrums around *T. scalaris*. This work underlines the urgent need for conservation measures since the habitats of these populations are increasingly threatened by human activities. By clarifying the taxonomy of these garter snakes, we aim to establish a foundation for future research and conservation efforts in the highlands of Jalisco.

Materials and methods

Taxonomic sampling

We collected multiple specimens of garter snakes of the genus *Thamnophis* from the highlands of Mexico between 2015 and 2022. We photographed all live snakes, including dorsal, lateral, and ventral profiles, and euthanized them with pentobarbital. We took tissue samples from muscle or liver upon death and preserved them in 96% ethanol. We fixed specimens in 10% formalin and transferred them to 70% ethanol for permanent storage.

All collected materials were deposited at the Instituto de Investigaciones sobre los Recursos Naturales (INIRENA) of the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH) in Morelia, Mexico; the Museo de Zoología, Facultad de Ciencias (MZFC) of the Universidad Nacional Autónoma de México (UNAM) in Mexico City; and the Facultad de Estudios Superiores, Zaragoza (MZFZ) of the Universidad Nacional Autónoma de México (UNAM), also in Mexico City. Museum acronyms throughout the text follow Sabaj (2020). Specimen numbers for all material examined are provided in Appendices 1, 2. We were not able to measure type specimens of previously described taxa, so we used measurements of the type specimens provided in original descriptions and other published literature (Cope 1861, 1866; Jan 1863; Boulenger 1893; Smith 1942; Walker 1955; Rossman 1969; Rossman and Burbrink 2005). Measurements for *T. errans* were taken from Webb (1976), whereas measurements from *Thamnophis sumichrasti* Cope, 1866, and *Thamnophis mendax* Walker, 1955, were taken from their original descriptions.

Distribution maps were generated based on the GBIF database (www.gbif.org), which includes both museum records and distribution records from the citizen scientist platform iNaturalist (inaturalist.org). iNaturalist records were curated by us to assure that no misidentifications were present before generating the maps and are current up through January 2024. Observations that could not be positively identified were removed.

The mountains of central Jalisco have numerous other species of *Thamnophis* that occur in sympatry or near sympatry with the species described herein. These include *Thamnophis copei* (Dugés, 1879), *Thamnophis cyrtopsis* (Kennicott, 1860), *Thamnophis eques* (Reuss, 1834), *Thamnophis melanogaster* (Peters, 1864), and *Thamnophis pulchrilatus* (Cope, 1885). While these species are not closely related to the species described herein, we include them in our comparatives within the species description to aid in the identification of individuals in the field.

Measurements and character states

Abbreviations used in the text and tables are as follows: snout–vent length (**SVL**), tail length (**TL**), total length (**TotL**), head length (**HL**), head width (**HW**), eye diameter (**ED**), rostral height (**RH**), rostral width (**RW**), internasal length (**INL**), internasal width (**INW**), prefrontal length (**PFL**), prefrontal width (**PFW**), frontal length (**FL**), maximum anterior frontal width (**MAFW**), maximum posterior frontal width (**MPFW**), parietal width (**PW**), parietal length (**PL**), loreal length (**LL**), loreal height (**LH**), mental length (**ML**) mental width (**MW**), anterior chin shield length (**ACSL**), posterior chin shield length (**PCSL**), internasal suture length (**INK**), prefrontal suture length (**PFK**), internasal rostral contact (**INR**), rostral nasal contact (**NR**), distance from frontal to snout (**DFS**), muzzle length (**MZL**), number of labials in contact with anterior chin shields (**LCAC**), ventral scales (**VS**) (Dowling (1951) method and explained in Rossman et al. (1996), subcaudal scales (**SC**), dorsal scale rows at one head length behind parietal (**DSRA**), dorsal scale rows at midbody (**DSRM**), dorsal scale rows at one head length prior to anal scale (**DSRT**), supralabials (**SL**), infralabials (**IL**), preoculars (**PRO**), postoculars (**POO**), maxilliary teeth (**MT**).

Scale measurements were taken in the following manner: **HL**, distance from the tip of the snout to the posterior border of the parietal scales; **HW**, distance taken at the posterior edge of the jaw; **RH**, the distance of the rostral scale from the median point of the mouth to the vertex formed by the internasal suture; **RW**, distance of the rostral scale measured between each suture formed by the 1st supralabial and prenasal; **INL**, distance of the internasal scale from its anterior border with the prenasal and rostral to its posterior border with the prefrontal; **INW**, distance from the prenasal to the medial suture between each internasal; **PFL**, distance from the postnasal, nasal, and internasal borders back to the border between the frontal and supraocular; **PFW**, distance from the postnasal to the median suture between the prefrontals; **FL** maximum length of the frontal shield; distance from the posterior part of the prefrontals to the medial union between the parietals; **MAFW**, the maximum width of the anterior portion of the frontal scale measured between each vertex formed by the prefrontal and supraocular scales; **MPFW**, the maximum width of the posterior portion of the frontal scale measured between each vertex formed by the parietal and supraocular scales, **PW**, distance from the union of the postocular and anterior temporal scales to the median suture between the parietals; **PL**, distance from the border between the supraocular and frontal to the posteriormost point of each parietal scale; **LL**, maximum length from the upper anterior border of the nasal to the lower posterior border with the preocular and supralabial; **LH**, distance from the supralabial to the union with the prefrontal and preocular; **ML**, taken from the medial point of the mouth to the posterior end of the mental scale, where the first pair of infralabials meet each other; **MW**, measured along the border of the mouth from one

infralabial border to the other; **ACSL**, distance from the suture between the first and second infralabial posteriorly to the median suture between posterior chinshields; **PCSL**, maximum length from the union with the anterior chinshield and infralabial to the posterior border of the posterior chinshield; **SC**, counted on the left and right sides, with the first subcaudal scale interpreted as the first scale posterior to the cloaca that was not counted by the anal scale; Dorsal scales were counted at one head length behind the posterior edge of the parietals, at midbody, and at one head length before the anterior border of the anal scale. Muzzle length was defined as the combined length of the internasal suture (**INK**) and pre-frontal suture (**PFK**); muzzle shape was calculated by dividing INR by NR.

DNA extraction, amplification, and molecular analyses

All laboratory procedures were carried out at the UNAM FES-Zaragoza in Mexico City. We used a standard ammonium acetate protocol (Fetzner 1999) to extract genomic DNA from liver or muscle tissue. We then sequenced two mitochondrial loci: Cytochrome b (Cytb) and NADH dehydrogenase subunit 4 (ND4). For Cytb, we used Gludg-L (Palumbi 1996) as the forward primer and ATRCB3 (Harvey et al. 2000) as the reverse primer. For ND4, we employed ND4 and ND4_Leu (Arevalo et al. 1994) as the forward and reverse primers, respectively. Both loci were amplified using a standard polymerase chain reaction (PCR) protocol: an initial denaturation at 95 °C for 3 minutes and 30 seconds, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 53 °C for 30 seconds, extension at 72 °C for 1 minute, and a final extension at 72 °C for 15 minutes, with a terminal hold at 10 °C. The PCR products were purified using a polyethylene glycol method (Lis 1980) and sequenced by Macrogen Korea (Standard-Seq of Macrogen Inc.).

Raw chromatograms were trimmed and edited using Geneious v. 2023.1 (Biomatters Ltd., Auckland, NZ). To infer phylogenetic relationships from the new samples, we included additional sequences of the genus *Thamnophis* as well as two outgroups obtained from GenBank. All new sequences were deposited in GenBank (Appendix 2).

Each gene was aligned separately in MAFFT version 7 (Katoh et al. 2017) using the Q-INS-I option. The alignments were then concatenated using FASconCAT v.1.04 (Kück and Longo 2014). The final alignment comprised 1828 base pairs (1104 bp for Cytb and 724 bp for ND4), including sequences from 81 representatives of the genus *Thamnophis* and individuals each of "*Adelophis*" *foxi* Rossman & Blaney, 1968 and "*Adelophis*" *copei* (Dugès, 1879), as well as one individual of *Nerodia erythrogaster* (Forster, 1771) and *Tropidoclonion lineatum* (Hallowell, 1856) as outgroups. We calculated pairwise genetic distances in the mitochondrial, Cytb, and ND4 genes using MEGA X software (Kumar et al. 2018).

Maximum likelihood (ML) analysis of the concatenated dataset was performed using IQ-TREE (Nguyen et al. 2015) using the IQ-TREE web server (Trifinopoulos et al. 2016). We employed an auto-substitution model and conducted 1,000 bootstrap replicates for support assessment. Separate ML analyses were also performed for each mitochondrial gene. The resulting topologies are presented in Suppl. materials 1, 2.

For Bayesian phylogenetic inference (BI), we initially used PartitionFinder v1.1.1 (Lanfear et al. 2012) to determine the most suitable model of partitions and nucleotide evolution for each locus using the Bayesian information criterion (BIC). The identified optimal partitions were: GTR + I + gamma for the first codon position of both Cytb & ND4, HKY + I + gamma for the second codon position of Cytb, HKY + gamma for the second codon position of ND4, and GTR + gamma for the third codon position of both Cytb & ND4. Our dataset was organized accordingly by locus and codon position. We then conducted BI using Mr. Bayes v3.2.2 (Ronquist et al. 2012) on the CIPRES science gateway server (Miller et al. 2011). This analysis involved four runs, each with 10 million generations and a sampling interval of 1,000 generations, incorporating three heated chains and one cold chain. Convergence was assessed using Tracer v1.6 (Rambaut et al. 2015), focusing on likelihood and parameter estimate overlaps, effective sample sizes, and the potential scale reduction factor (PSRF). Convergence was achieved within 200,000 generations, allowing us to discard the initial 25% of each run as burn-in. The results of these runs were combined using TreeAnnotator 2.7.4. (Bouckaert et al. 2019) to create a concatenated tree and visualized with FigTree v1.4.2 (Rambaut 2014).

Considering that the topologies from both ML and BI analyses were almost identical, we have included only the maximum likelihood phylogeny in this paper, with the Bayesian Inference phylogeny provided as Suppl. material 3.

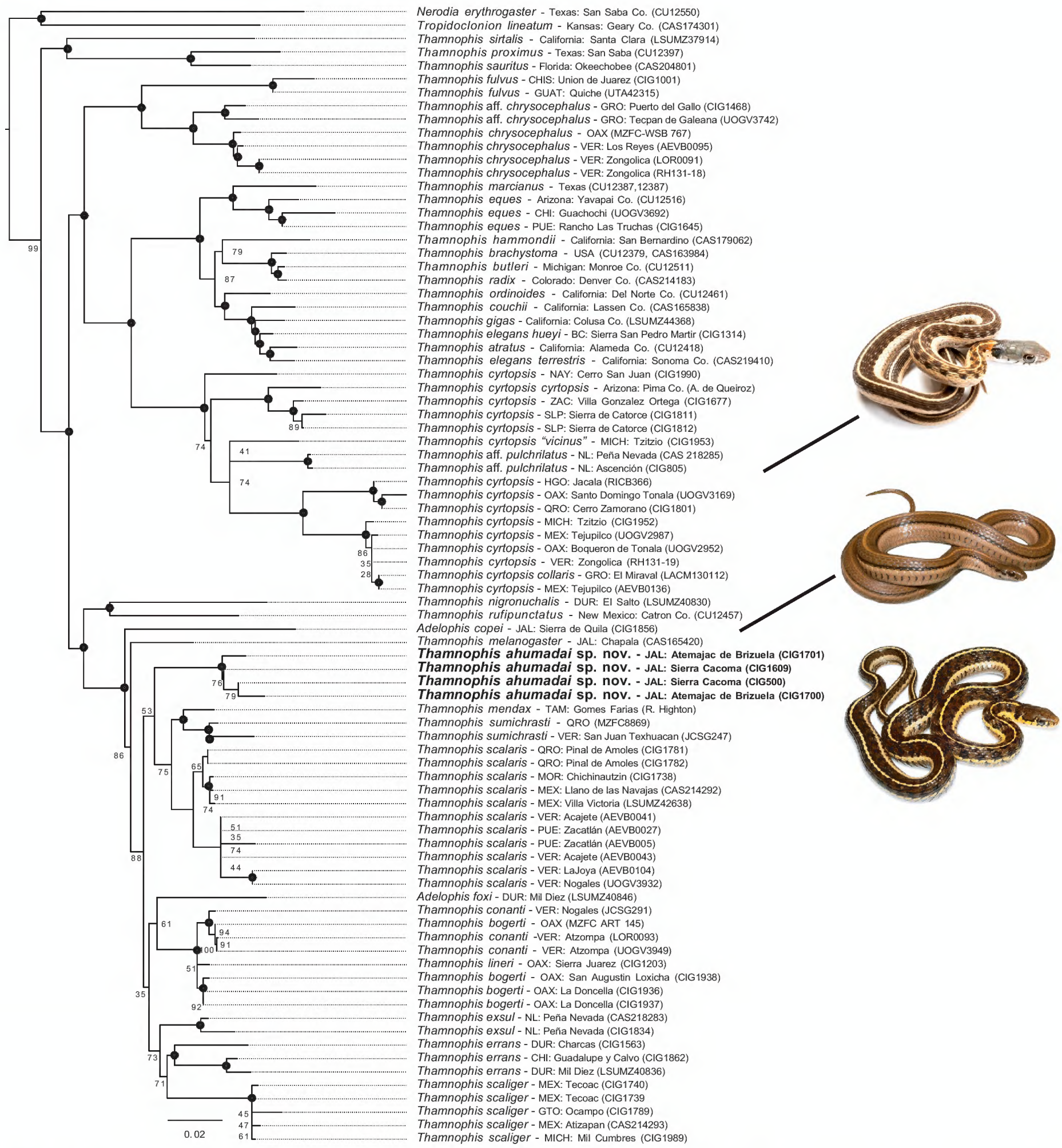
Results

Molecular phylogenetic results

In our ML phylogeny (Fig. 1), we find concordance with prior evolutionary hypotheses, including those derived from mitochondrial DNA (mtDNA) studies (de Queiroz et al. 2002), those combining mtDNA and nuclear DNA in their analyses (McVay et al. 2015, Deepak et al. 2022), as well as analyses by Hallas et al. (2022), which were based on ddRADseq data. The robustness of our phylogenetic nodes is largely high, with most nodes garnering greater than 95% bootstrap support. However, several internal nodes exhibited lower support values, so we collapsed nodes below the 50% bootstrap support threshold.

Our analysis corroborates the delineation of three primary clades within *Thamnophis*, aligning with pre-

vious studies. The first clade, "Ribbon Snakes," is composed of *Thamnophis sirtalis* (Linnaeus, 1758), *Thamnophis proximus* (Say, 1823), and *Thamnophis saurita* (Linnaeus, 1766), collectively forming a lineage sister to the remaining members of *Thamnophis*. The other sampled *Thamnophis* segregate into two major clades. The first of these, referenced as the "Widespread Clade" by de Queiroz et al. (2002), predominantly comprises species from the USA and northern Mexico. Our data support the close phylogenetic relationship between *Thamnophis fulvus* (Bocourt, 1893) and *Thamnophis chrysocephalus* (Cope, 1885), albeit with notable genetic divergence among *T. chrysocephalus* populations from Guerrero compared to those from Oaxaca and Veracruz. Further, the clade encompassing *T. fulvus* and *T. chrysocephalus* formed a sister lineage to a cluster of species endemic to the USA and northern Mexico, with *Thamnophis. cyrtopsis* forming a sister relationship to a clade comprising species from the USA and Baja California, inclusive of "*T.* aff. *pulchrilatus*" and an individual matching the original description of *Thamnophis vicinus* Smith, 1942 (currently a junior synonym of *T. cyrtopsis*), which is notable as this population has never been studied phylogenetically.


Nerodia erythrogaster - Texas: San Saba Co. (CU12550)
Tropidoclonion lineatum - Kansas: Geary Co. (CAS174301)
Thamnophis sirtalis - California: Santa Clara (LSUMZ37914)
Thamnophis proximus - Texas: San Saba (CU12397)
Thamnophis sauritus - Florida: Okeechobee (CAS204801)
Thamnophis fulvus - CHIS: Union de Juarez (CIG1001)
Thamnophis fulvus - GUAT: Quiche (UTA42315)
Thamnophis aff. chrysocephalus - GRO: Puerto del Gallo (CIG1468)
Thamnophis aff. chrysocephalus - GRO: Tecpan de Galeana (UOGV3742)
Thamnophis chrysocephalus - OAX (MZFC-WSB 767)
Thamnophis chrysocephalus - VER: Los Reyes (AEVB0095)
Thamnophis chrysocephalus - VER: Zongolica (LOR0091)
Thamnophis chrysocephalus - VER: Zongolica (RH131-18)
Thamnophis marcianus - Texas (CU12387,12387)
Thamnophis eques - Arizona: Yavapai Co. (CU12516)
Thamnophis eques - CHI: Guachochi (UOGV3692)
Thamnophis eques - PUE: Rancho Las Truchas (CIG1645)
Thamnophis hammondii - California: San Bernardino (CAS179062)
Thamnophis brachystoma - USA (CU12379, CAS163984)
Thamnophis butleri - Michigan: Monroe Co. (CU12511)
Thamnophis radix - Colorado: Denver Co. (CAS214183)
Thamnophis ordinoides - California: Del Norte Co. (CU12461)
Thamnophis couchii - California: Lassen Co. (CAS165838)
Thamnophis gigas - California: Colusa Co. (LSUMZ44368)
Thamnophis elegans hueyi - BC: Sierra San Pedro Martir (CIG1314)
Thamnophis atratus - California: Alameda Co. (CU12418)
Thamnophis elegans terrestris - California: Sonoma Co. (CAS219410)
Thamnophis cyrtopsis - NAY: Cerro San Juan (CIG1990)
Thamnophis cyrtopsis cyrtopsis - Arizona: Pima Co. (A. de Queiroz)
Thamnophis cyrtopsis - ZAC: Villa Gonzalez Ortega (CIG1677)
Thamnophis cyrtopsis - SLP: Sierra de Catorce (CIG1811)
Thamnophis cyrtopsis - SLP: Sierra de Catorce (CIG1812)
Thamnophis cyrtopsis "vicinus" - MICH: Tzitzio (CIG1953)
Thamnophis aff. pulchrilatus - NL: Peña Nevada (CAS 218285)
Thamnophis aff. pulchrilatus - NL: Ascención (CIG805)
Thamnophis cyrtopsis - HGO: Jacala (RICB366)
Thamnophis cyrtopsis - OAX: Santo Domingo Tonala (UOGV3169)
Thamnophis cyrtopsis - QRO: Cerro Zamorano (CIG1801)
Thamnophis cyrtopsis - MICH: Tzitzio (CIG1952)
Thamnophis cyrtopsis - MEX: Tejupilco (UOGV2987)
Thamnophis cyrtopsis - OAX: Boqueron de Tonala (UOGV2952)
Thamnophis cyrtopsis - VER: Zongolica (RH131-19)
Thamnophis cyrtopsis collaris - GRO: El Miraval (LACM130112)
Thamnophis cyrtopsis - MEX: Tejupilco (AEVB0136)
Thamnophis nigronuchalis - DUR: El Salto (LSUMZ40830)
Thamnophis rufipunctatus - New Mexico: Catron Co. (CU12457)
Adelophis copei - JAL: Sierra de Quila (CIG1856)
Thamnophis melanogaster - JAL: Chapala (CAS165420)
Thamnophis ahumadai sp. nov. - JAL: Atemajac de Brizuela (CIG1701)
Thamnophis ahumadai sp. nov. - JAL: Sierra Cacoma (CIG1609)
Thamnophis ahumadai sp. nov. - JAL: Sierra Cacoma (CIG500)
Thamnophis ahumadai sp. nov. - JAL: Atemajac de Brizuela (CIG1700)
Thamnophis mendax - TAM: Gomes Farias (R. Highton)
Thamnophis sumichrasti - QRO (MZFC8869)
Thamnophis sumichrasti - VER: San Juan Texhuacan (JCSG247)
Thamnophis scalaris - QRO: Pinal de Amoles (CIG1781)
Thamnophis scalaris - QRO: Pinal de Amoles (CIG1782)
Thamnophis scalaris - MOR: Chichinautzin (CIG1738)
Thamnophis scalaris - MEX: Llano de las Navajas (CAS214292)
Thamnophis scalaris - MEX: Villa Victoria (LSUMZ42638)
Thamnophis scalaris - VER: Acajete (AEVB0041)
Thamnophis scalaris - PUE: Zacatlán (AEVB0027)
Thamnophis scalaris - PUE: Zacatlán (AEVB005)
Thamnophis scalaris - VER: Acajete (AEVB0043)
Thamnophis scalaris - VER: LaJoya (AEVB0104)
Thamnophis scalaris - VER: Nogales (UOGV3932)
Adelophis foxi - DUR: Mil Diez (LSUMZ40846)
Thamnophis conanti - VER: Nogales (JCSG291)
Thamnophis bogerti - OAX (MZFC ART 145)
Thamnophis conanti -VER: Atzompa (LOR0093)
Thamnophis conanti - VER: Atzompa (UOGV3949)
Thamnophis lineri - OAX: Sierra Juarez (CIG1203)
Thamnophis bogerti - OAX: San Augustin Loxicha (CIG1938)
Thamnophis bogerti - OAX: La Doncella (CIG1936)
Thamnophis bogerti - OAX: La Doncella (CIG1937)
Thamnophis exsul - NL: Peña Nevada (CAS218283)
Thamnophis exsul - NL: Peña Nevada (CIG1834)
Thamnophis errans - DUR: Charcas (CIG1563)
Thamnophis errans - CHI: Guadalupe y Calvo (CIG1862)
Thamnophis errans - DUR: Mil Diez (LSUMZ40836)
Thamnophis scaliger - MEX: Tecoac (CIG1740)
Thamnophis scaliger - MEX: Tecoac (CIG1739
Thamnophis scaliger - GTO: Ocampo (CIG1789)
Thamnophis scaliger - MEX: Atizapan (CAS214293)
Thamnophis scaliger - MICH: Mil Cumbres (CIG1989)
99
79
87
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41
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86
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28
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75
65
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74
51
35
74
44
88
61
94
91
100
51
35
92
73
71
45
47
61
0.02

Figure 1. Concatenated maximum likelihood inference of the phylogenetic relationships of *Thamnophis* and closely related Natricines based on the mitochondrial genes Cytb and ND4.

In the other major clade, referenced as the "Mexican Clade," our results recover an early split between *T. nigronuchalis* Thompson, 1957 + *T. rufipunctatus* (Cope, 1875), sister to all other lineages. This split is followed by the divergence of *T. copei* and *T. melanogaster*. Remarkably, this study represents the first inclusion of *T. copei* in a molecular phylogeny, revealing an unexpected non-sister relationship to *T. foxi*, which was its sole congener in the now invalid genus *Adelophis* (see below). The new species reported herein formed a polytomy with *T. mendax*, *T. sumichrasti*, and *T. scalaris*. That group was recovered as the sister clade to a group that includes *Thamnophis exsul* Rossman, 1969; *T. errans* + *T. scaliger*; and a group comprising *T. foxi* alongside *T. bogerti* Rossman & Burbrink, 2005; *T. conanti* Rossman & Burbrink, 2005; and *T. lineri* Rossman & Burbrink, 2005. However, it is noteworthy that the support for this latter grouping is very low, and *T. bogerti*, *T. conanti*, and *T. lineri* do not form monophyletic groups.

Finally, our BI analysis exhibited a high degree of similarity to those relationships obtained through maximum likelihood, with an almost identical topology. The key distinction lies in the variation of support values for certain groups. In the BI analysis, *T. ahumadai* was recovered as a sister to *T. mendax* + *T. sumichrasti* + *T. scalaris*, but with low support (posterior probability = 0.61). The BI tree, elucidating these differences, is presented in Suppl. material 3.

Our molecular results support the hypothesis that populations formerly assigned to *Thamnophis scalaris* from Jalisco, Mexico, belong to an undescribed species. We analyzed molecular samples from two isolated highland populations (Sierra Cacoma, Sierra de Tapalpa; Appendix 2) of "*T. scalaris*" from Jalisco. Both are each other's closest relatives, with 100% bootstrap support. These populations fall within the "Mexican Clade" of our phylogenetic tree (Fig. 1). Together, these two populations are sister to a clade comprising *T. scalaris*, *T. sumichrasti*, and *T. mendax*, albeit with low bootstrap support (67%). The two Jalisco populations have similar genetic distances to their closest relatives. These genetic distances are 0.04–0.05 (ND4) and 0.03 (Cytb) to *Thamnophis bogerti* (as understood herein, see below), which appears to be their closest relative according to genetic distances. The two Jalisco populations also have genetic distances of 0.04–0.06 (ND4) and 0.03–0.04 (Cytb) from the superficially similar *T. scalaris*; 0.05–0.07 (ND4) and 0.04–0.05 (Cytb) from geographically proximate populations of *T. errans*; and 0.04–0.05 (ND4) and 0.04 (Cytb) from *T. exsul*. In comparison, the *T. scalaris* populations analyzed herein (Morelos, Estado de México, Querétaro, Puebla, and Veracruz) have intraspecific genetic distances of 0.00–0.02 (ND4) and 0.00–0.01 (Cytb). Within the *T. scalaris* populations analyzed, it should be noted that one specimen (AEVB 0104) from La Joya, Acajete, Veracruz had a ND4 genetic distance of 0.02–0.04 from other non-Veracuz specimens of *T. scalaris*. Similarly, another specimen (UOGV 3932) from nearby Nogales, Veracruz, had a higher Cytb genetic distance (0.02) from all other specimens of *T. scalaris*. These genetic distances are still low when compared to the interspecific genetic distances that we recovered between other species, but they are nonetheless interesting and may show evidence of hybridization with nearby populations of *T. bogerti* (see below).

Systematic account

Thamnophis ahumadai sp. nov.

https://zoobank.org/3D2B6CF0-C0D6-443C-B7A4-3AF2DC32AF77

Figs 2, 4, 5, 7a–e

Proposed standard English name. Ahumada's Alpine Garter Snake.

Proposed standard Spanish name. Culebra Listonada de Montaña de Ahumada.

Type materials. ***Holotype*** (Fig. 2). INIRENA 2933 (original field number CIG-1612). Adult male collected under a log in a marshy meadow near a pond, 2.8 km E of Cumbre de Guadalupe, Municipio de Cuautla, Jalisco, Mexico (20.168991, -104.684925, 2,353 m asl; datum = WGS84; Fig. 4a), by Christoph I. Grünwald, María del Carmen G. Mendoza-Portilla, Iván Ahumada-Carrillo, and Ginny N. Weatherman on 23 August 2019.

Paratypes (Fig. 3). MZFZ 4593 (original field number CIG-0500) adult male collected under a rock on a hillside above a stream at Cumbre de Guadalupe, Municipio de Talpa de Allende, Jalisco, Mexico (20.168902, -104.711252, 2,137 m asl; datum = WGS84) by Christoph I. Grünwald, Héctor Franz-Chávez, André J. Grünwald, Ámbar Lanomy Grünwald, Janelle Morales-Flores, and Karen I. Morales-Flores on 11 July 2015. MZFZ 4594 (original field number CIG-1609) adult female collected under trash in a grassy meadow amongst pine forest, 1.5 km N of Cumbre de Guadalupe, Municipio de Talpa de Allende, Jalisco, Mexico (20.186581, -104.716188, 2,179 m asl; datum = WGS84), by Christoph I. Grünwald, María del Carmen G. Mendoza-Portilla, Iván Ahumada-Carrillo, and Ginny N. Weatherman on 23 August 2019. INIRENA 2932 and 2934 (original field numbers CIG-1611 and CIG-1613) adult male and adult female, respectively, with the same collection data as Holotype. MZFZ 4595 (original field number CIG-1610) adult male collected under rock in meadow, 4.2 km airline ESE of Cumbre de Guadalupe, Municipio de Tomatlán, Jalisco, Mexico (20.157217, -104.675059, 2,319 m asl; datum = WGS84), by Christoph I. Grünwald, María del Carmen G. Mendoza-Portilla, Iván Ahumada-Carrillo, and Ginny N. Weatherman on 23 August 2019. INIRENA 2935–36 (original field number CIG-1700–01) 2 adult males, collected under logs at a large pond at 2.5 km SE of Atemajac de Brizuela, Municipio de Atemajac de Brizuela, Jalisco, Mexico (20.1151, -103.7036, 2,422 m asl; datum = WGS84; Fig. 4b), by Christoph I. Grünwald, André J. Grünwald, and Ámbar Lanomy Grünwald on 21 June 2020.

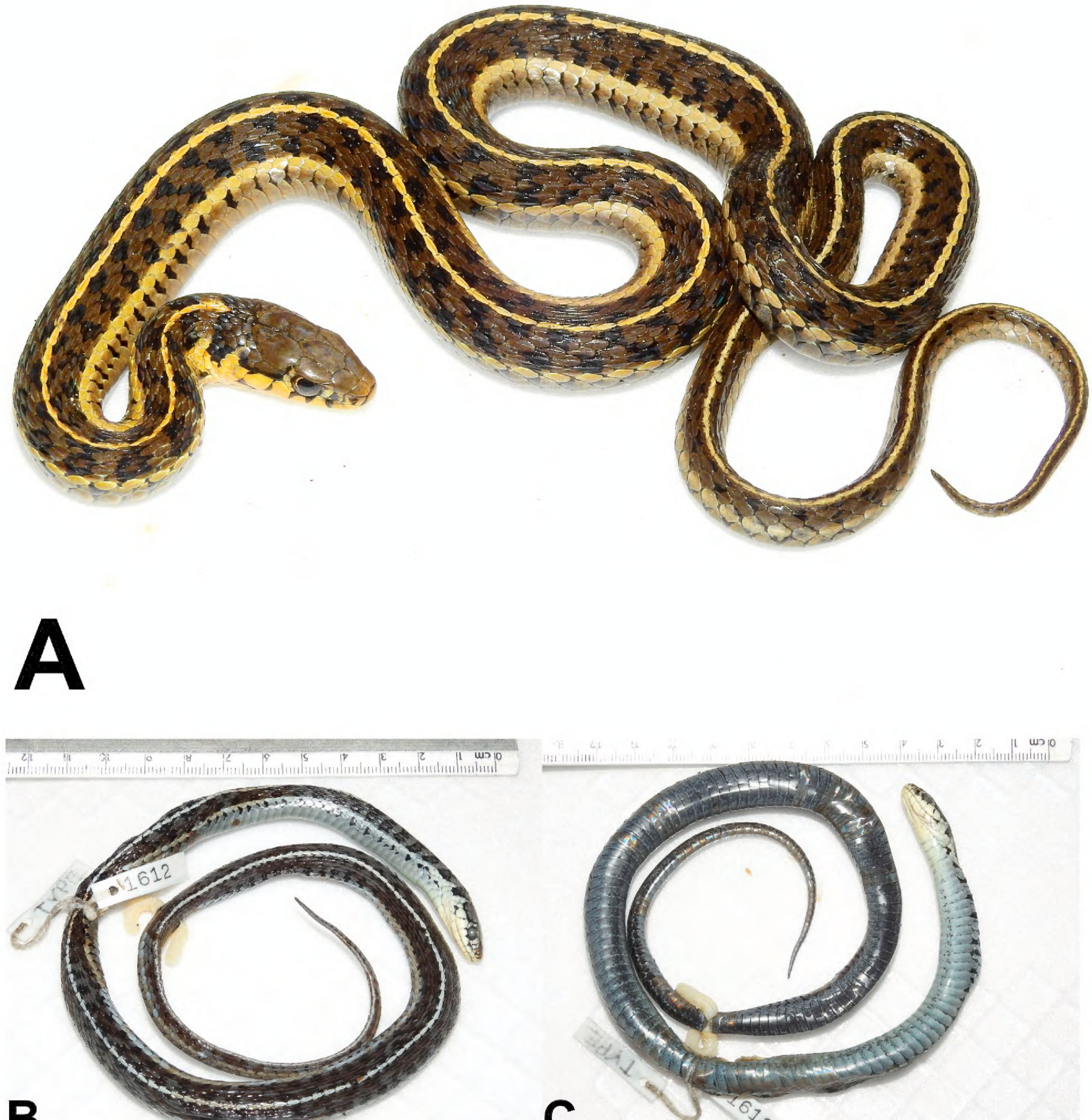

Figure 2. *Thamnophis ahumadai* sp. nov. holotype (INIRENA 2933) from the vicinity of Cumbre de Guadalupe, in the Municipio de Cuautla, Jalisco, Mexico.

Diagnosis. A relatively small Garter Snake, with a maximum of 565 mm SVL and 705 TotL; head narrow, scarcely wider than neck, with a short muzzle (INK + PFK = 3.0 mm); tail long in length, 31–36% of SVL and 23–27% of TotLin males, 25–26% of SVL and 20–21% of TotLin females; internasals wider than long; frontal 2–2.25 times longer than wide; loreal slightly longer than wide; one preocular; 2–4 postoculars; temporals 1 + 2; 7 supralabials, third and fourth entering orbit; 9–10 infralabials, first four in contact with anterior chinshields; anterior chinshields shorter than posterior. Dorsal scales always in 19-17-17 rows, strongly keeled except for the outermost row, which is smooth; ventral scales in males 134–142, in females 139–141; subcaudals in males 61–69, in females 54–55; anal scale undivided. Dorsal pattern consists of a pale mid-vertebral stripe restricted to the mid-dorsal row and a pale lateral stripe on second scale row. One or two rows of dark brown or black dorso-lateral spots, usually in one row on the anterior third of the dorsum, then divided into two rows along the remaining posterior two thirds. Ventral coloration dark, pale anteriorly, becoming progressively darker until dark gray or black posteriorly.

Figure 3. *Thamnophis ahumadai* sp. nov. paratypes. **A.** Male (INIRENA 2932) from same locality as holotype; **B.** Male (INIRENA 2936) from 2.5 km SE of Atemajac de Brizuela, Municipio de Atemajac de Brizuela, Jalisco, Mexico; **C.** Male (MZFZ 4595) from 4.2 km airline ESE of Cumbre de Guadalupe, Municipio de Tomatlán, Jalisco, Mexico; **D.** Male (INIRENA 2935) from 2.5 km SE of Atemajac de Brizuela, Municipio de Atemajac de Brizuela, Jalisco, Mexico; **E.** Male (MZFZ 4593) from Cumbre de Guadalupe, Municipio de Talpa de Allende, Jalisco, Mexico.

Figure 4. Collection localities for *Thamnophis ahumadai* sp. nov., including the type locality at 2.8 km E of Cumbre de Guadalupe, Municipio de Cuautla, Jalisco, Mexico (**A**) and 2.5 km SE of Atemajac de Brizuela, Municipio de Atemajac de Brizuela, Jalisco, Mexico (**B**).

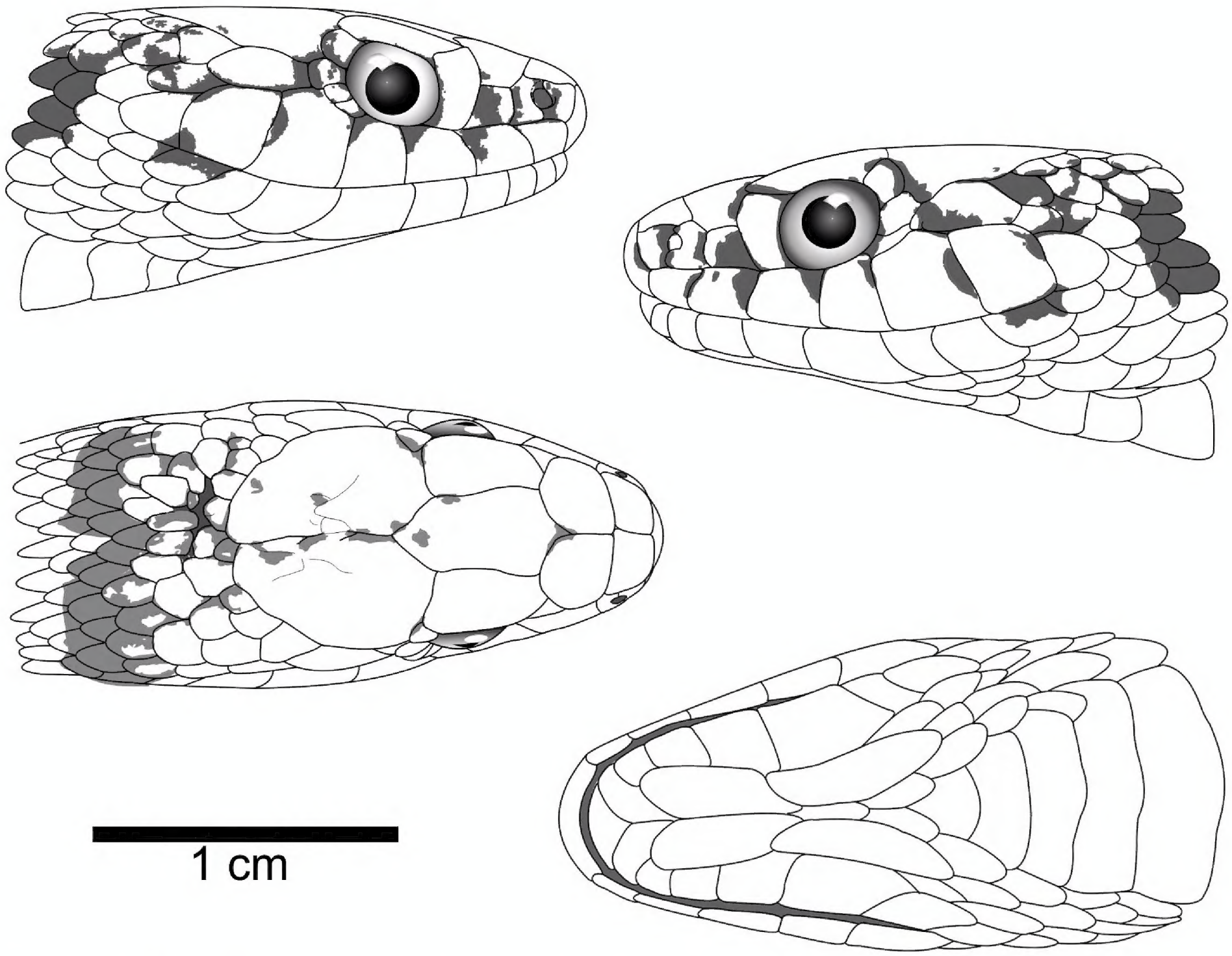

Figure 5. Head drawings of the holotype of *Thamnophis ahumadai* sp. nov. (INIRENA 2933). A solid line depicts 1 cm. Drawings by Iván T. Ahumada-Carrillo.

Comparisons. *Thamnophis ahumadai* can be distinguished from *T. scalaris* (comparison character traits in parenthesis) by possessing 19-17-17 dorsal scale rows (vs. 17-17-17); 61–69 subcaudals in males (vs. 69–77, but see below for Querétaro population); a shorter tail length in males, 23–27% TL/Totl ratio (vs. 26–33%) and also in females, 20–21% (vs. 20–25%); a black nuchal blotch (vs. brown); two rows of dark brown or black dorsal blotches between mid-dorsal and dorso-lateral pale stripes (vs. one row of large brown dorsal blotches); and a dark gray to black ventral coloration on latter two thirds of body (vs. ventral coloration same as darker portions of lateral coloration).

Thamnnophis ahumadai can be distinguished from *T. errans* (*T. errans* character traits in parenthesis) by possessing 134–142 ventral scales in males (vs. 150–166) and 139–141 ventral scales in females (vs. 146–160); 61–

69 subcaudals in males (vs. 78–94) and 54–55 subcaudals in females (vs. 67–83); a shorter tail length in females, 20–21% TL/TotL ratio (vs. 23–25%); a dorsal pattern with at least some larger dorsal spots fusing together towards the anterior portion of the body (vs. all dorsolateral spots in two alternating rows); and by possessing small black spots on the lateral pale stripe (vs. black spotting absent on lateral pale stripe).

Thamnophis ahumadai can be distinguished from *T. godmani* (*T. godmani* character traits in parenthesis) by possessing 19-17-17 dorsal scale rows (vs. 17-17-17); 54–69 subcaudals in males (vs. 74–88) and 54–55 subcaudals in females (vs. 61–77); a shorter tail length in males, 23–27% TL/TotL (vs. 25–29%), and also in females, 20–21% (vs. 25–27%). *Thamnophis ahumadai* can be distinguished from *T. bogerti* (*T. bogerti* character traits in parenthesis) by possessing 19-17-17 dorsal scale rows (vs. 17-17-15), 134–142 ventral scales in males (vs. 135–157); 54–55 subcaudal scales in females (vs. 56–68). Moreover, *Thamnophis ahumadai* is distinguished from both species by possessing two rows of alternating dark brown or black blotches between the mid-dorsal stripe and lateral stripes on dorsum (vs. two rows of dark spots between mid-dorsal stripe and lateral stripe may be absent, limited to randomly occurring black scale outlines, or consist of small black spots).

Thamnophis ahumadai can be distinguished from *T. scaliger* (*T. scaliger* character traits in parenthesis) by possessing supralabials that are all entirely pale-colored, with black pigment along each scale suture (vs. several posterior supralabials darkly pigmented by the encroachment of an occipital blotch, anterior ground color of anterior supralabials pale); 61–69 subcaudal scales in males (vs. 49–58), and 54–55 subcaudal scales in females (vs. 40–49); a longer tail length in males, 23–27% TL/Totl ratio (vs. 17–21%) and in females 20–21% TL/TotL (vs. 16–19%); no pale coloration along head scale sutures (vs. pale coloration present along head scale sutures); a black nuchal blotch (vs. brown); and two rows of dark brown or black dorsal blotches between the mid-dorsal and dorso-lateral pale stripes (vs. one row of large brown dorsal blotches).

Thamnophis ahumadai can be distinguished from *T. exsul* (*T. exsul* character traits in parenthesis) by possessing 19-17-17 dorsal scale rows (vs. 17-17-17); 134–142 ventral scales in males (vs. 142–150) and 139–141 ventral scales in females (vs. 152–156); and a pale mid-dorsal stripe always present (vs. absent or only present anteriorly).

Thamnophis ahumadai can be distinguished from both *T. mendax* and *T. sumichrasti* by possessing 134–142 ventral scales in males (vs. 145–149 in *T. mendax* and 151–161 in *T. sumichrasti*); from *T. sumichrasti* by possessing 139–141 ventral scales in females (vs. 147–159) and by possessing 61–69 subcaudals in males (vs. 72–78); from *T. mendax* by possessing 54–55 subcaudal scales in females (vs. 56–60) and by shorter tail in females 20–21% TL/TotL (vs. 21–23%); from both *T. mendax* and *T. sumichrasti* by possessing a lateral pale stripe (vs. absent); furthermore *T. ahumadai* does not present two pattern classes, and can readily distinguished from the blotched pattern class of *T. mendax* and both pattern classes of *T. sumichrasti* by possessing a pale mid-dorsal stripe (vs. lacking in both species).

The mountains of central Jalisco have numerous other species of *Thamnophis* that occur in sympatry or near sympatry with *T. ahumadai*. These species (except *T. copei*) are all distantly related to *T. ahumadai* and can be readily distinguished by their appearance. *Thamnophis ahumadai* differs from *T. cyrtopsis*, *T. eques*, and *T. pulchrilatus* by having 17 dorsal scale rows at mid-body (vs. 19 or more) and by having a tongue that is black (vs. red with black tips). From the apparently closely related *T. copei* (see below), *T. ahumadai* differs by possessing a loreal (vs. fused with prefrontal), 17 dorsal scale rows (vs. 15), and a longer head with seven supralabials (vs. 5).

For comparative purposes, we include photographs of *T. ahumadai* and closely related species in Fig. 6 and at a higher resolution in Suppl. material 5.

Description of holotype (Fig. 2). All measurements in mm. An adult male, 357 SVL, 122 TL, 479 TotL, tail relatively long, with TL/ SVL ratio 0.34 and TL/TotL ratio 0.25. Head short, twice as long as wide, and HL/ SVL 0.04. All dorsal scales keeled except in first lateral row, dorsal scales in 19 rows anteriorly, reducing to 17 at the 21st ventral scale row and then 17 throughout rest of body. Ventral scales 135, anal scale undivided, 62/63 divided subcaudal scales. Rostral wider (3.2) than high (2.3); internasals wider (2.2/2.1) than long (1.7); prefrontal subequal, slightly longer (2.7/2.6) than wide (2.5/2.6); frontal 5.4 long, anterior edge 4.0 from snout; frontal 2.7 wide anteriorly and 2.5 wide posteriorly; parietal longer (6.3/6.1) than wide (3.4/3.5); loreal slightly longer (1.7/1.6) than wide (1.3); seven supralabials on each side and nine infralabials on each side. One preocular on each side and ¾ postoculars. Eye relatively small, ED 3.2, and ED/HL ratio 0.22. Mental wider (2.4) than long (1.25); anterior chin shields (4.8/4.3), in contact with 4 infralabials on each side; posterior chin shields longer than anterior chin shields (5.1/5.0). For a detailed drawing of the head scalation, see Fig. 5.

Everted hemipenes are the length of seven subcaudals; they are long and narrow with no noticeable widening in the apical region.

Coloration in preservative (Fig. 2b, c). Head scales brown, but frontal, supraoculars, and parietals pale gray, apparently damaged as the snake was pre-ecdysis. Supralabials cream-white, with black lines along sutures. Dark nuchal blotch 1–3 scales long, black in coloration, complete dorsally from below the labial region on both sides. Pale mid-dorsal stripe white, starting on the fourth dorsal scale posterior to the parietals and running to the tip of the tail. One row of large dorso-lateral blotches, nine on the left, six on the right; large blotches divided into two rows of alternating dorso-lateral blotches. Lateral pale stripe bluish-gray, one second and third scale rows on the neck, and then limited to the second scale row on the rest of body and tail, slowing fading in intensity on tail. A single, lateral row of black spots present on the first dorsal scale row,

Figure 6. Photographs of species related to *Thamnophis ahumadai* sp. nov. in life, all from Mexico. *Thamnophis ahumadai* sp. nov. (**A**) from Cumbre de Guadalupe, Municipio de Talpa de Allende, Jalisco; *Thamnophis ahumadai* sp. nov. (**B**) from 2.8 km E of Cumbre de Guadalupe, Municipio de Cuautla, Jalisco; *Thamnophis ahumadai* sp. nov. (**C**) 4.2 km airline ESE of Cumbre de Guadalupe, Municipio de Tomatlán, Jalisco; *Thamnophis ahumadai* sp. nov. (**D**) from 2.5 km SE of Atemajac de Brizuela, Municipio de Atemajac de Brizuela, Jalisco; *Thamnophis ahumadai* sp. nov. (**E**) from Tapalpa, Municipio de Tapalpa, Jalisco; *Thamnophis ahumadai* sp. nov. (**F**) from 2.5 km SE of Atemajac de Brizuela, Municipio de Atemajac de Brizuela, Jalisco; *Thamnophis errans* (**G**) from vicinity of Los Amoles, Sierra Huichol, Jalisco; *Thamnophis errans* (**H**) from Los Charcos, Municipio de Mezquital, Durango; *Thamnophis errans* (**I**) from Los Charcos, Municipio de Mezquital, Durango; *Thamnophis errans* (**J**) from 28 km SSW of Tepehuanes, Municipio de Tepehuanes, Durango; *Thamnophis errans* (**K**) from La Catedral, Municipio de Guadalupe y Calvo, Chihuhua *Thamnophis exsul* (**L**) from Peña Nevada, Nuevo León (photo by Robert Hansen) *Thamnophis scalaris* (**M**) from Volcán Chichinautzin, Municipio de Huitzilac, Morelos; *Thamnophis scalaris* (**N**) from Villa Victoria, Municipio de Villa Victoria, Estado de México (photo by Hugo Plata-Tinoco); *Thamnophis scalaris* (**O**) from Huamantla, Municipio de Huamantla, Tlaxcala (photo by Anibal Díaz de la Vega Pérez); *Thamnophis scalaris* (**P**) from Pico de Orizaba, Veracruz (photo by Alfredo Gutiérrez); *Thamnophis scalaris* (**Q**) from 1 km N of Pinal de Amoles, Municipio de Pinal de Amoles, Quéretaro; *Thamnophis scalaris* (**R**) from 1 km N of Pinal de Amoles, Municipio de Pinal de Amoles, Quéretaro; *Thamnophis godmani* (**S**) from San Vicente, Municipio de Chilpancigo de los Bravo, Guerrero; *Thamnophis godmani* (**T**) from San Vicente, Municipio de Chilpancigo de los Bravo, Guerrero; *Thamnophis bogerti* (**U**) from La Doncella, Municipio de San Mateo Río Hondo, Oaxaca; *Thamnophis bogerti* (**V**) from Sierra Miahuatlán, Oaxaca; *Thamnophis bogerti* (**W**) from Llano de las Flores, Sierra Juárez, Oaxaca; *Thamnophis bogerti* (**X**) from Puerto del Aire, Municipio de Acultzingo, Veracruz.

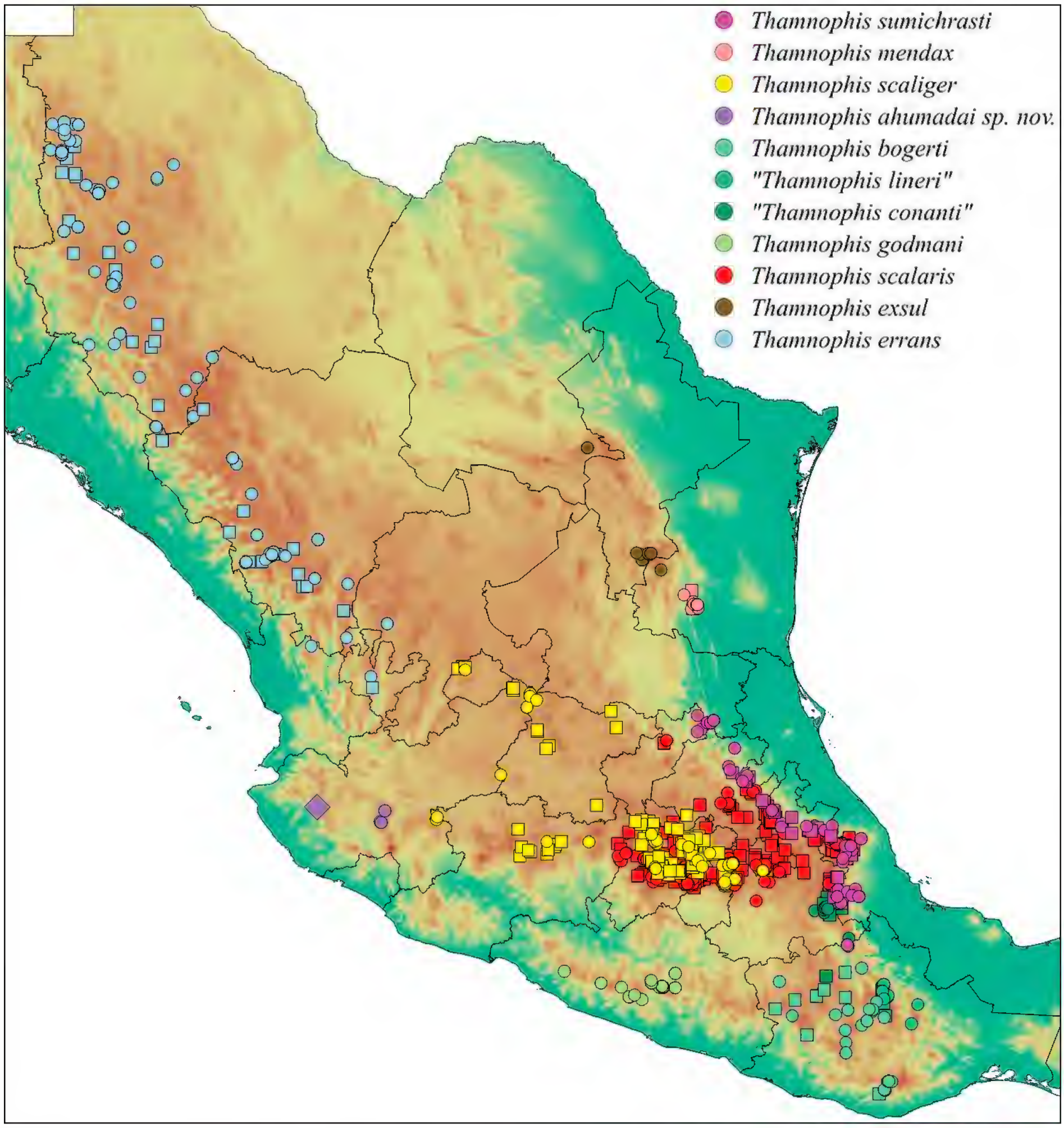

Figure 7. Distribution map of snakes similar to *Thamnophis scalaris* in Mexico. Circles represent museum records; squares represent verified iNaturalist observations, or field observations made by us but without a specimen deposited in a collection. The diamond represents the type locality of *Thamnophis ahumadai* sp. nov. We have included the different populations of *Thamnophis bogerti* in the key with their former names in quotation marks, and we used different shades of green to depict their individual ranges.

spots about half a scale in size on the anterior quarter of the body, then reducing to about a third a scale in size on the posterior three quarters of the body. Ventral surfaces of head and throat creamy-white until the fourth ventral scale, becoming pale bluish gray until approximately the 50th ventral scale, then progressively turning darker until the 78th ventral scale, where the venter takes on a dark gray-black coloration that continues posteriorly to the tip of the tail.

Coloration in life (Fig. 2a). Dorsal coloration chocolate brown, with 80 pairs of black dorso-lateral blotches present on dorsum. Pale mid-dorsal stripe present, pale yellow on anterior half of body, and then fading to a cream pigment posteriorly until the tail tip. Pale stripe involving only the vertebral row of scales. Top of head brown, including parietals, frontal, supraoculars, prefrontals and internasals, with rostral and front part of internasal pale cream, similar to supralabials. Supralabials pale cream on first three supralabials, then pale yellow on the latter four supralabials. Supralabials 2–6 have a black outline along the upper posterior suture. Nasal cream below, brown above, with a black outline posteriorly. Loreal anterior half cream, posterior half black. Single preocular cream;

four postoculars also cream; upper one with black outline on posterior border. Anterior temporal brown above and black below; posterior temporals brown and black. A bilobed dark nuchal spot present behind head, dark brown anteriorly, and black posteriorly. Nuchal spot three scales long at mid-dorsal line, extending ventrally to the height of the jawline. This nuchal blotch partially divides the pale-yellow coloration on the posterior supralabials and the pale lateral stripe on the lower portion of the dorsum. Pale lateral stripe on first three scale rows anteriorly, pale yellow, then restricted to second scale row on the anterior four-fifths of the dorsum, only slightly involving the first and third scale rows. After the anterior fifth of the dorsum, the first scale row is cream with dark black markings on the posterior edge of each scale, giving the appearance of small black vertical blotches. Tail same color as posterior body, black above, cream below, with a pale cream mid-dorsal stripe and small black spots on the cream colored first scale row. Iris copper.

Measurements (mm). SVL 357; TL 122 mm; TotL; 479; HL 14.; HW 7.3; ED 3.2 mm; RH 2.3; RW 3.2; INL 1.7; INW 2.1; PFL 2.7/2.6; PFW2.5/2.6; FL 5.4; MAFW 2.7; MPFW 2.4; PL 6.2/6.1; PW 3.4/3.5; LL 1.6; LH 1.3; ML 1.25; MW 2.4; ACSL 4.8/4.3; PCSL 5.1/5.0.

Variation. Meristic variation is minimal amongst the paratypes. Specimen ranged in size from 255 mm (INIRENA 2935) to 565 mm (INIRENA 2934). The shortest relative tail length was 0.20 TL/TotL in INIRENA 2934, whereas the longest relative tail length was 0.27 in INIRENA 2932. Interestingly, both were males from the same locality. Most all specimens had more ventral scales than the holotype, with MZFZ 4595 possessing the highest count (142). The ventral scale + subcaudal scale combinations amongst specimens ranged from 194 (INIRENA 2934) to 210/211 (MZFZ 4595). No variation in number of dorsal scale rows or supralabials was documented. Two specimens (INIRENA 2934, 2936) had 10 infralabials on both sides. Postoculars usually three on at least one side, but MZFZ 4593 has 4/4 and MZFZ 4594 has 4/6. The two females (INIRENA 2934, MZFZ 4594) have a head HL/HW ratio of 1.1–1.4, whereas the males have a HL/HW ratio of 1.5–2.0. Color pattern variation exists. The vertebral stripe ranges from orange (INIRENA 2932), yellow or yellowish (INIRENA 2936, MZFZ 4595), to cream (MZFZ 4593, INIRENA 2935). MZFZ 4593 is unique in that it presents dorsolateral blotches that are fused, similar to *T. scalaris* and *T. scaliger*. INIRENA 2934 has a dark dorsal coloration, which makes the dorsal pattern barely visible and gives the snake a dark, unpatterned appearance. Morphological and meristic variation of the all available specimens, including the holotype and all paratypes is given in Table 1.

Distribution and habitat. This species appears to be restricted to grasslands and meadows in pine-oak woodland and pine forest above 2100 m asl. Only known from two mountain ranges in Jalisco, the Cumbre de Guadalupe region of the Sierra Cacoma, and in the vicinities of the towns of Atemajac de Brizuela and Juanacatlán in the Sierra de Tapalpa. This species has been collected at elevations ranging from 2140 to 2450 m asl. We have included a range map with known localities of this species and closely related species in Fig. 7.

Etymology. A patronym honoring Iván Trinidad Ahumada-Carrillo (1984–), who has made many contributions to diverse areas in herpetology, including extensive studies of the herpetofauna of Jalisco and Zacatecas. Iván collected the first specimen of this new species in the Sierra Cacoma (MZFZ 4593) and pointed out its distinctiveness from typical *T. scalaris* and *T. errans*.

Conservation. This species of garter snake is only known from two relatively small high-elevation areas in the highlands of Jalisco, which fall within the "Jaliscan Transverse Range Pine-Oak Woodland (42)" and "Jaliscan Sierra Madre del Sur Mixed Temperate Woodland (46)" biogeographical formations as mapped by Grünwald et al. (2015). Due to its small distribution, we recommend that this species be rewarded with the highest level of protection possible from the Mexican government. As habitat destruction such as logging and farming is ongoing in both localities where it occurs, this species may qualify as Endangered under the IUCN criterion. However, it can also be considered to be in the DD (data deficient) category due to uncertainties about whether or not this species occurs in Michoacán (see below). More fieldwork should be done to determine the full extent of the distribution of *T. ahumadai* and whether or not it is present in other highland regions of Jalisco or adjacent Michoacán.

Discussion

With the description of *T. ahumadai*, we remove *T. scalaris* from the herpetofauna of Jalisco. We also suggest that all specimens of "*T. scalaris*" collected from Jalisco (NHMUK 92.9.5.39, 92.10.31.20–24; UTA 4040, 4932–47, 4949, 5991–93; KU 87472–73) are assignable to *T. ahumadai*. A detailed morphological examination of these specimens will undoubtedly expand the known variation in this newly described species. Boulenger (1893) gave a detailed description of specimens of "*T. scalaris*" from Jalisco. The combination of 7 supralabial scales and low ventral and subcaudal scale counts that he reported precludes their assignment to any other species of *Thamnophis* known from Jalisco except for *T. scaliger*. While we cannot definitely exclude *T. scaliger* from the description given by Boulenger, it is important to note that despite intensive recent collecting at both localities (Cumbre de Guadalupe and Atemajac de Brizuela), no *T. scaliger* has been collected from either of these mountain ranges. We tentatively suggest that these specimens are referrable to *T. ahumadai*. Smith et al. (1950) reviewed these same specimens, but they confused them with their concept of "*T. scalaris scaliger*."

Apparently, *T. ahumadai* and *T. scalaris* are widely isolated along the Mexican Transverse Ranges, with no known records between the Sierra Tapalpa in central Jalisco and the closest populations occurring near

Table 1. Morphometric and meristic variation of *Thamnophis ahumadai* sp. nov. Measurements of the holotype are shaded gray.

	MZFZ 4593	MZFZ 4594	MZFZ 4595	INIRENA 2932	INIRENA 2933	INIRENA 2934	INIRENA 2935	INIRENA 2936
Sex	Male	Female	Male	Male	Male	Female	Male	Male
SVL	262	426	297	335	357	565	255	327
TL	86	112	103	122	122	140	78	114
TotL	348	538	400	457	479	705	333	441
TL/SVL	0.33	0.26	0.35	0.36	0.34	0.25	0.31	0.35
TL/TotL	0.25	0.21	0.26	0.27	0.25	0.20	0.23	0.26
VS	140	141	142	135	135	139	134	139
SC	68/68	54/54	68/69	67/67	62/63	55/55	61/62	64/65
VS+SC	208	195	210.5	203	197.5	194	195.5	203.5
DSRA	19	19	19	19	19	19	19	19
DSRM	17	17	17	17	17	17	17	17
DSRT	17	17	17	17	17	17	17	17
SL	7/7	7/7	7/7	7/7	7/7	7/7	7/ 7	7/7
IL	9/9	9/9	9/9	9/10	9/9	10/10	9/9	10/10
PRO	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
POO	4/4	4/6	3/3	3/3	3/4	3/3	3/3	3/3
ED	2.61	3.96	3.11	3.15	3.19	4.50	3.01	3.54
ED/HL	0.21	0.21	0.24	0.21	0.22	0.20	0.24	0.26
HL	12.44	18.59	12.70	15.08	14.43	22.90	12.56	13.65
HW	8.24	13.34	8.59	8.96	7.34	20.54	7.70	8.74
HL/HW	1.51	1.39	1.48	1.68	1.97	1.11	1.63	1.56
HL/SVL	0.05	0.04	0.04	0.05	0.04	0.04	0.05	0.04
MAFW	2.65	3.41	2.39	3.63	2.71	4.58	2.53	2.92
MPFW	2.17	2.91	2.25	2.62	2.46	3.54	1.84	2.24
PFK	1.6	2.39	1.82	2.12	1.67	2.15	1.69	1.74
INK	1.21	2.07	1.42	1.44	1.31	*	1.29	1.52
RH	2.41	2.93	2.12	2.48	2.27	3.46	1.58	2.59
RW	3.4	4.41	3.17	3.53	3.23	5.87	3.03	3.47
INL	1.61 / 1.48	2.69/2.82	1.62/ 1.61	1.67/ 1.66	1.71/ 1.66	*	1.52/ 1.75	1.66/ 1.43
INW	1.86 / 1.73	2.55/ 2.58	1.88/ 1.81	2.21/ 2.08	2.08/ 2.11	*	1.68/ 1.79	1.89/ 2.21
PFL	2.31 / 2.36	3.49/ 3.52	2.38/ 2.30	2.73/ 2.82	2.71/ 2.64	3.49/ 3.39	2.31/ 2.38	2.54/ 2.66
PFW	2.43 / 2.39	3.35/ 3.23	2.46/ 2.56	2.66/ 2.68	2.51/ 2.59	4.25/ 4.46	2.12/ 2.18	2.58/ 2.36
FL	4.92	6.57	4.76	5.44	5.41	8.34	4.58	4.93
PL	5.41 / 5.15	7.51/7.22	5.20/ 5.33	5.74/ 5.88	6.27/ 6.11	9.30/ 9.55	4.95/ 5.28	5.72/ 5.95
PW	3.43 / 3.69	2.67/2.68	3.57/ 3.63	3.79/ 3.94	3.43/ 3.49	5.38/ 5.24	3.22/ 3.35	3.87/ 3.55
DFS	3.89	5.32	4.04	4.56	3.95	7.27	4.14	4.12
LL	1.47/ 1.46	2.49/ 2.56	1.48/ 1.39	1.69/ 1.61	1.68/ 1.62	2.61/ 2.53	1.20/ 1.38	1.34/ 1.43
LH	1.27/ 1.23	1.83/ 1.76	1.64/ 1.68	1.26/ 1.29	1.28/ 1.29	2.30/ 2.23	1.15/ 1.10	1.26/ 1.21
ML	1.22	1.81	1.21	1.38	1.25	2.43	0.88	1.35
MW	2.08	2.84	2.34	2.73	2.36	3.83	2.01	2.45
LCAC	4/4	4/4	4/4	4/5	4/4	5/5	4/4	5/5
ACSL	4.0 / 4.05	5.89/ 5.62	3.85/ 4.10	4.49/ 4.43	4.77/ 4.34	7.98/ 8.50	3.50/ 3.57	4.21/ 4.0
PCSL	4.10 / 3.99	6.62/ 6.32	5.02/ 4.57	4.88/ 4.74	5.11/ 5.03	10.02/ 9.24	4.51/ 4.71	5.65/ 6.10

the vicinity of Nahuatzen in central Michoacán (Rossman et al. 1996; Rossman and Lara-Gongora 1997). The Nahuatzen population is of special interest itself. The Nahuatzen area is a particularly high elevation (ca. 3000 m) upland situated in the middle of a continuous swath of moderate mountains (ca. 2000 m) in central Michoacán. While this region is not particularly isolated, the nearest collecting localities of highland *Thamnophis* are *T. ahumadai*, 190 km to the west at Atemajac de Brizuela, Jalisco, and *T. scalaris*, 170 km to the east near Zitacuaro, Michoacán. Based on color pattern alone, these snakes seem very similar to and may be conspecific with *T. ahumadai*. We were unable to acquire molecular data from this population as all specimens in Mexican collections are fixed in formalin. Field work should be done around Nahuatzen to determine whether this population represents an isolated population of *T. ahumadai*, an isolated population of *T. scalaris*, or another yet undescribed species of *Thamnophis*.

Monophyly of *Thamnophis scaliger* and *Thamnophis scalaris*

Based on eleven genetic samples of *Thamnophis scalaris* collected across its range and an additional five samples of *Thamnophis scaliger* from several localities in central Mexico, we found that both *T. scalaris* and *T. scaliger* are monophyletic and do not represent sister species (Fig. 1). De Queiroz et al. (2002) had confusing results for the relationship between *T. scalaris* and *T. scaliger* based on the placement of their "*T. scaliger* 2" within *T. scalaris*. Their "*T. scaliger* 2" was based on LSUMZ 42638 (Accession Number: AF420189), which is supposedly a *T. scaliger* from the vicinity of Villa Victoria, Estado de México. We compared this sample to five *T. scaliger* samples and nine *T. scalaris* samples, all identified in the field by us. This sample was consistently recovered in a clade containing morphologically verified *T. scalaris* in all of our analyses. While we were not able to examine the specimen, we hy-

pothesize that this genetic sample was misidentified as a *T. scaliger* by de Quieroz et al. (2002) and consequently re-identify it as *T. scalaris*.

The positioning of the former genus *Adelophis* within *Thamnophis*

The genus *Adelophis* Dugès *in* Cope, 1879, was originally described as a close relative of the North American genus *Tropidoclonion* Cope, 1860. The first authors to suggest a close relationship between *Adelophis* and *Thamnophis* were Dunn (1931) and Rossman and Blaney (1968), who suggested that *Adelophis* was derived from *Thamnophis*. Later, de Queiroz et al. (2002) obtained molecular sequences from a specimen of *Adelophis foxi* (LSUMZ 40846) and found that it grouped within *Thamnophis*, with its closest relatives being *T. melanogaster* and *T. validus*. De Queiroz et al. (2002) suggested that their sample of *Adelophis* may have been mixed up with another sample; however, they assessed that the genetic distances of the analyzed sequences were too far removed from any of the other analyzed species to represent any of them. These authors had samples from all valid Mexican species of *Thamnophis* at that time except for two (*Thamnophis postremus* Smith, 1942, and *Thamnophis rossmani* Conant, 2000) and cautioned against making any taxonomical changes pending further sampling of "*A. foxi*" and the similar "*A. copei.*" McVay et al. (2015) obtained additional sequences from the same specimen of "*A. foxi*" and confirmed the results obtained by de Queiroz et al. (2002), thus dispelling the possibility of PCR contamination. Nonetheless, these authors also refrained from making any taxonomical changes because the sequenced specimen was the same individual used earlier by de Queiroz et al. (2002) and was thus subject to the same caveats as the prior study. Later, Hallas et al. (2022) found *Adelophis* to be placed within *Thamnophis* across three molecular datasets (mtDNA, nDNA, and ddRADseq) and formally sank the genus *Adelophis* into *Thamnophis*. They cautioned that "some might refrain from formal changes to *A. copei* until that taxon can be suitably evaluated in a phylogenetic analysis." However, Hallas et al. (2022) considered the putative sister relationship between "*A. copei*" and "*A. foxi*" (Rossman and Blaney 1968; Rossman and Wallach 1987) to be sufficient evidence refuting the argument that "*A. copei*" could be nested outside of *Thamnophis*. In this study, we sequenced samples from a specimen of *Thamnophis copei* for the first time and confirmed that *T. copei* falls within the "southern clade" of *Thamnophis* like *T. foxi* (de Queiroz et al. 2002; McVay et al. 2015; Hallas et al. 2022). This confirms that the placement of *Thamnophis copei* is correct and that *Adelophis* should be subsumed into *Thamnophis*. Surprisingly, however, our results suggest that the morphologically similar *T. copei* and *T. foxi* are not sisters to one another, though both are consistently nested within the "Mexican clade" of *Thamnophis*. More extensive sequence data is needed to determine the exact phylogenetic relationship between these two species and closely related species. It is noteworthy that *T. foxi* has not been collected since the 1970s, despite several recent collection attempts.

The validity of the species related to *Thamnophis godmani* in the Sierra Madre del Sur

Thamnophis godmani was described from Omiltemi and "Amula" in Guerrero (Günther 1886) and was later considered to be distributed in the Sierra Madre del Sur and Sierra Madre Oriental from central Guerrero and central Veracruz (respectively) to the Isthmus of Tehuantepec (Rossman et al. 1996). *Thamnophis godmani* has historically been recognized as a close relative of *T. scalaris*, and Smith (1942) even considered *T. godmani* to be a subspecies of the former taxon. The specific identity of *T. godmani* was reaffirmed when more specimens and morphological data became available (Rossman in Varkey 1979). Later, Rossman and Burbrink (2005) conducted a multivariate study of morphological characters in various populations of *T. godmani* and described three species: *T. bogerti*, *T. conanti*, and *T. lineri*.

Rossman and Burbrink (2005) provided subtle morphological and mensural characters to justify the description of *T. bogerti*, *T. conanti*, and *T. lineri* as species distinct from *T. godmani*. While the morphological data provided does suggest that *T. godmani* may be specifically distinct from the other three, the morphological differences are trivial once data from *T. bogerti*, *T. conanti*, and *T. lineri* are compared to one another. When compared to the intraspecies variation of other widespread highland *Thamnophis* such as *T. scaliger*, *T. scalaris*, *T. errans*, *T. chrysocephalus*, and *T. sumichrasti*, the subtle geographic variation found across *T. bogerti* and its relatives over a relatively broad range suggests that these taxa likely represent a single evolutionary species unit. Morphological and mensural differences for the three species were presented in the description only in text; however, here we compare them in Table 2.

We evaluated the status of these species (*T. bogerti*, *T. conanti*, and *T. lineri*) in our phylogeny, and our results indicate that *T. bogerti* is paraphyletic with respect to *T. conanti* and *T. lineri* (Fig. 1, Suppl. materials 1–3). Additionally, we found very low levels of genetic divergence between these species. Genetic distances in the mitochondrial gene ND4 were as low as 0.002 between *T. bogerti* and *T. conanti*, whereas both of these species show a genetic distance of 0.04–0.05 with their closest relative, *T. scalaris*. Genetic distances in the Cytb between *T. bogerti*, *T. conanti*, and *T. lineri* ranged from 0.00–0.01, whereas all three of these "species" had distances between 0.03–0.04 from their closest relative, *T. ahumadai*. (Suppl. material 4). These results support the tree topologies of McVay et al. (2015) and

Table 2. Comparisons of *Thamnophis godmani*, *T. bogerti*, *T. conanti*, and *T. lineri* taken from the descriptions of the latter three (Rossman and Burbrink 2005).

	T. godmani	*T. bogerti*	*T. conanti*	*T. lineri*
DSR	maximum DSR 17	maximum DSR 17	maximum DSR 17	maximum DSR 17
Maxillary Teeth	Maxillary Teeth 17–21	Maxillary Teeth 17–20	Maxillary Teeth 16–18	Maxillary Teeth 18–20
Dorsal Head Coloration	top of head unpatterned	top of head unpatterned	top of head unpatterned	top of head unpatterned
Dorsal Pattern	two rows of small black spots between light lines	two rows of small black spots between light lines	two rows of small black spots between light lines	two rows of small black spots between light lines
Nuchal Blotch Coloration	nuchal blotch coloration black	nuchal blotch coloration variable, only 15% brown	nuchal blotches brown	nuchal blotches predominately brown
Suture coloration on SL	prominence of black bar along posterior suture of SL 5 equal to or greater than bar along SL 6 and SL 7 suture	prominence of black bar along posterior suture of SL 5 equal to or less than bar along SL 6 and SL 7 suture	prominence of black bar along posterior suture of SL 5 equal to or less than bar along SL 6 and SL 7 suture	black bar along posterior suture of SL 5 reduced or absent
VS	V 144 males, 138 in females	V 145 males, 140 females	V 150 in males, 144 females	140 in males, 136 in females
SC	SC average 79 in males, 71 females	SC average 70 males, 62 females	SC average 72 males, 62 females	SC average 62 males, 55 females
TL/TotL	TL/TotL 27% males, 26% females	TL/TotL 25% males, 23% females	TL/TotL 25% males, 23% females	TL/TotL 23.5% males, 21.5% females
Prefrontal Length	prefrontal suture usually slightly shorter than internasal suture PFK/INK 94%	prefrontal suture usually slightly longer than internasal suture PFK/INK 106%	prefrontal suture usually slightly longer than internasal suture PFK/INK 105%	prefrontal suture usually slightly longer than internasal suture PFK/INK 106%
Muzzle Shape	muzzle tip very broad, INR / NR 134%	muzzle tip usually broad, INR / NR 115%	muzzle tip broad INR / NR 107%	muzzle tip usually broad, INR / NR 116%
Nasal Condition	anterior and posterior nasal subequal	anterior nasal usually shorter than posterior nasal	anterior nasal usually shorter than posterior nasal	anterior nasal usually shorter than posterior nasal
Parietal Length	parietal short FL/PL 88%	FL/PL 77%	FL/PL 77%	FL/PL 80%
Frontal Condition	Frontal Broad Posteriorly / MPFW/ MAFW 72%	Frontal Broad Posteriorly / MPFW/ MAFW 79%	Frontal Broad Posteriorly / MPFW/ MAFW 75%	Frontal Broad Posteriorly / MPFW/ MAFW 85%

Hallas et al. (2022). Unfortunately, we did not have any genetic material of *T. godmani* to compare with, and recent analyses that did include *T. godmani* (Hallas et al. 2022) are based on a sample of *T. bogerti* from Oaxaca (J. Campbell, pers. comm). *Thamnophis godmani* is not known to occur in Oaxaca, and all samples of "*T. godmani*" found on Genbank are based on specimens that were collected before *T. bogerti* was described (Rossman and Burbrink 2005). Consequently, our phylogenetic results indicate that *T. bogerti*, *T. conanti*, and *T. lineri* belong to the same evolutionary unit. As these three species were described in the same paper, we invoke Article 24.2 of the International Code of Zoological Nomenclature (ICZN 1999) and suggest that *T. lineri* and *T. conanti* are junior synonyms of *T. bogerti*, the latter of which was alphabetically and sequentially described first in Rossman and Burbrink (2005). The specific relationship between *T. godmani* and *T. bogerti* remains to be tested; however, the isolated nature of the Guerrero populations of this species may indeed prove to be of a specific nature.

Rossman and Burbrink (2005), in their review of the populations formerly assigned to *T. godmani*, stated that "*T. godmani* occurs in at least four discrete geographic areas that are effectively separated at the present time by habitat disjunctions unsuitable for these residents of montane pine-oak forests (1768–3048 m)." However, these sky islands inhabited by the snakes formerly assigned to *T. godmani* have not been proven to act as biogeographic barriers for other reptiles (Bryson et al. 2011; Palacios-Aguilar et al. 2021). Furthermore, *T. bogerti* is frequently collected as low as 1100–1200 m in humid environs in Oaxaca, and oak woodland in the Sierra Juárez and Sierra Zongolica ranges down to at least 1500 m. Thus, these perceived "isolated mountain ranges" are not barrier-isolated for an elevation-adapted snake like *T. bogerti*.

Identity of *Thamnophis* similar to *T. scalaris* in the Sierra de Pinal de Amoles, Querétaro

An unidentified population of *Thamnophis* occurs in the vicinity of Pinal de Amoles in the Sierra Gorda region of Querétaro (Rossman and Lara-Gongora, 1997). These specimens (UMMZ 105415–416) are cataloged as *T. scalaris* but originate from well outside of the known range of this species. Rossman and Lara-Gongora (1997) discussed these specimens in detail, comparing them to *T. scalaris*, *T. scaliger*, and *T. exsul*, and concluded that "until fresh material becomes available, it would seem prudent to defer judgment of the identity of these Querétaro specimens." We collected two specimens (INIRENA 2937–38, Fig. 8) of this population in 2020 and included them in our analyses.

Morphologically, the two specimens seem to match *T. scalaris* relatively well. INIRENA 2937 is an adult female and has 17-17-17 dorsal scale rows, whereas INIRENA 2938 is a subadult male and has 19-17-17 dorsal scales. Supralabials are seven on both specimens; infralabials are 9/10 on INIRENA 2937 and 10/10 on INIRENA 2938. Ventrals 141 on both specimens, subcaudals 52 (INIRENA 2937) and 57 (INIRENA 2938), respectively. The subcaudal count is lower than the ranges given for eastern *T. scalaris* by Rossman and Lara-Gongora (1997), which were 53–69 (females) and 64–85 (males). The tail lengths are short for *T. scalaris*, with TL/TotL 19.8% on INIRENA 2937 and 21.4% on INIRENA 2938. Rossman and Lara (1997) gave TL/TotL ranges of 20.2–25.0% (females) and 25.9–33.2% (males). Rossman and Lara (1997) noted that the hemipenes on UMMZ 104415 were nine subcaudals long. The hemipenes on INIRENA 2938 are six subcaudals long, although they may not have been everted to their fullest extent. While the tail lengths

Figure 8. Photographs of *Thamnophis scalaris* from Querétaro. Female (INIRENA 2937) (**A, B**) and male (INIRENA 2938) (**C, D**) both from 1 km N of Pinal de Amoles, Municipio de Pinal de Amoles, Querétaro.

and subcaudal counts are very low for eastern *T. scalaris* and the hemipenes are short, in all other aspects, these specimens seem to match *T. scalaris* reasonably well.

Our phylogenetic analysis suggests that these specimens are nested with *T. scalaris*. Genetic distances for ND4 between this population and nearby Veracruz *T. scalaris* range from 0.00–0.01. In comparison, the genetic distances of ND4 between the Queretaro specimens and *T. scaliger* range from 0.02–0.06, between *T. exsul* 0.04 and *T. errans* 0.05. The genetic distance of Cytb in these specimens to nearby *T. scalaris* in Puebla and northern Veracruz ranges from 0.00–0.01. Comparatively, the genetic distances of Cytb in the Queretaro specimen to specimens of *T. scaliger* are 0.04–0.07, and for *T. exsul*, they are 0.04. Based on these results, we formally assign the Pinal de Amoles (Sierra Gorda) population to *T. scalaris* and confirm the presence of *T. scalaris* in Querétaro.

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Appendix 1

Table A1. Specimens examined. Two samples are from shed skins where specimens were not collected, but genetic material was included in the analysis and these are included in bold print.

Field number	Museum number	Date	Species	Locality	State	Coordinates	Elevation
CIG-1936	pending	12-Jun-21	*Thamnophis bogerti*	Municipio de San Mateo Río Hondo: La Doncella	Oaxaca	16.121632, -96.505225	2736 m
CIG-1937	pending	12-Jun-21	*Thamnophis bogerti*	Municipio de San Mateo Río Hondo: La Doncella	Oaxaca	16.121632, -96.505225	2736 m
CIG-1938	pending	13-Jun-21	*Thamnophis bogerti*	Municipio de San Augustín Loxicha: La Paz Obispo	Oaxaca	16.069370, -96.577196	2160 m
CIG-0626	pending	10-Oct-15	*Thamnophis cyrtopsis*	Municipio de Santiago: La Camotera, near Laguna de Sánchez	Nuevo León	25.318675, -100.212369	1583 m
CIG-0805	pending	28-May-16	*Thamnophis cyrtopsis*	Municipio de Aramberri: S of La Ascención	Nuevo León	22.22677, -99.87677	2010 m
CIG-1677	pending	NA	*Thamnophis cyrtopsis*	Municipio de Noria de Angeles: Villa de Gonzalez Ortega	Zacatecas	22.407739, -101.875726	2300 m
CIG-1801	pending	02-Aug-20	*Thamnophis cyrtopsis*	Municipio de Colón: Los Trigos, Cerro Zamorano	Querétaro	20.906709, -100.206763	2606 m
CIG-1811	pending	08-Aug-20	*Thamnophis cyrtopsis*	Municipio de Catorce: 11.3 km airline ESE of Estación Wadley	San Luis Potosí	23.577618, -100.878592	2794 m
CIG-1812	pending	08-Aug-20	*Thamnophis cyrtopsis*	Municipio de Catorce: 11.3 km airline ESE of Estación Wadley	San Luis Potosí	23.577618, -100.878592	2794 m
CIG-1990	pending	11-Jul-21	*Thamnophis cyrtopsis*	Municipio de Tepic: NE side of Rancho La Noria	Nayarit	21.4860, -104.9921	1572 m
CIG-1991	pending	11-Jul-21	*Thamnophis cyrtopsis*	Municipio de Tepic: NE side of Rancho La Noria	Nayarit	21.4860, -104.9922	1572 m
CIG-1992	pending	11-Jul-21	*Thamnophis cyrtopsis*	Municipio de Tepic: NE side of Rancho La Noria	Nayarit	21.4860, -104.9923	1572 m
CIG-1993	pending	11-Jul-21	*Thamnophis cyrtopsis*	Municipio de Tepic: NE side of Rancho La Noria	Nayarit	21.4860, -104.9924	1572 m
CIG-1994	pending	11-Jul-21	*Thamnophis cyrtopsis*	Municipio de Tepic: NE side of Rancho La Noria	Nayarit	21.4860, -104.9925	1572 m
CIG-1995	pending	11-Jul-21	*Thamnophis cyrtopsis*	Municipio de Tepic: NE side of Rancho La Noria	Nayarit	21.4860, -104.9926	1572 m
CIG-1996	pending	11-Jul-21	*Thamnophis cyrtopsis*	Municipio de Tepic: NE side of Rancho La Noria	Nayarit	21.4860, -104.9927	1572 m
CIG-1997	pending	11-Jul-21	*Thamnophis cyrtopsis*	Municipio de Tepic: NE side of Rancho La Noria	Nayarit	21.4860, -104.9928	1572 m
CIG-1998	pending	11-Jul-21	*Thamnophis cyrtopsis*	Municipio de Tepic: NE side of Rancho La Noria	Nayarit	21.4860, -104.9929	1572 m
CIG-1999	pending	11-Jul-21	*Thamnophis cyrtopsis*	Municipio de Tepic: NE side of Rancho La Noria	Nayarit	21.4860, -104.9930	1572 m
CIG-2000	pending	11-Jul-21	*Thamnophis cyrtopsis*	Municipio de Tepic: NE side of Rancho La Noria	Nayarit	21.4860, -104.9931	1572 m
CIG-2001	pending	11-Jul-21	*Thamnophis cyrtopsis*	Municipio de Tepic: NE side of Rancho La Noria	Nayarit	21.4860, -104.9932	1572 m
CIG-2002	pending	11-Jul-21	*Thamnophis cyrtopsis*	Municipio de Tepic: NE side of Rancho La Noria	Nayarit	21.4860, -104.9933	1572 m
CIG-2003	pending	11-Jul-21	*Thamnophis cyrtopsis*	Municipio de Tepic: NE side of Rancho La Noria	Nayarit	21.4860, -104.9934	1572 m
CIG-2004	pending	11-Jul-21	*Thamnophis cyrtopsis*	Municipio de Tepic: NE side of Rancho La Noria	Nayarit	21.4860, -104.9935	1572 m

Field number	Museum number	Date	Species	Locality	State	Coordinates	Elevation
CIG-2006	pending	06-Sep-21	*Thamnophis cyrtopsis*	Municipio de Yécora: 3.8 km W of Yécora on Hwy. 16	Sonora	28.3619, -108.96497	1691 m
CIG-2183	pending	18-Aug-22	*Thamnophis cyrtopsis*	Municipio de Tecpán de Galeana: La Laguna	Guerrero	17.577641, -100.767848	1055 m
CIG-2005	pending	06-Sep-21	*Thamnophis eques*	Municipio de Yécora: west of Yécora	Sonora	28.374691, -109.028245	1942 m
CIG-2138	pending	30-Jun-22	*Thamnophis eques*	Municipio de Aguascalientes: Hwy. 36, 1.2 km NW of Jalisco stale line.	Aguascalientes	21.720405, -102.155511	2007 m
CIG-1645	pending	06-Mar-19	*Thamnophis eques*	Municipio de Huachinango: Rancho Las Truchas	Puebla	20.114709, -98.107585	2041 m
CIG-1646	pending	06-Mar-19	*Thamnophis eques*	Municipio de Huachinango: Rancho Las Truchas	Puebla	20.114709, -98.107585	2041 m
CIG-1562	pending	08-Aug-19	*Thamnophis errans*	Municipio de Mezquital: Los Charcos	Durango	23.012354, -104.299608	2693 m
CIG-1563	pending	08-Aug-19	*Thamnophis errans*	Municipio de Mezquital: Los Charcos	Durango	23.012354, -104.299608	2693 m
CIG-1564	pending	08-Aug-19	*Thamnophis errans*	Municipio de Mezquital: Los Charcos	Durango	23.012354, -104.299608	2693 m
CIG-1862	pending	26-Oct-20	*Thamnophis errans*	Municipio de Guadalupe y Calvo: Terreros	Chihuahua	26.194814, -106.588628	2590 m
CIG-2239	pending	30-Jun-22	*Thamnophis errans*	Municipio de Aguascalientes: Hwy. 36, 1.2 km NW of Jalisco stale line.	Aguascalientes	25.066337, -106.319703	2537 m
CIG-1833	**NA**	**10-Aug-20**	***Thamnophis exsul***	**Cerro Peña Nevada, Puerto Peña Nevada**	**Nuevo León**	**23.823812, -99.878490**	**2657 m**
CIG-1834	**NA**	**10-Aug-20**	***Thamnophis exsul***	**Cerro Peña Nevada, salamander spot 2**	**Nuevo León**	**23.836308, -99.856751**	**2658 m**
CIG-1001	pending	06-Sep-16	*Thamnophis fulvus*	Puente Malá, El Chiquihuite, Unión Juárez	Chiapas	15.093985, -92.091741	1790 m
CIG-1468	pending	29-Jun-19	*Thamnophis* aff. *chrysocephalus*	just below Puerto del Gallo	Guerrero	17.48739, -100.19932	2457 m
CIG-1315	pending	23-Apr-18	*Thamnophis hammondii*	Cienega La Grulla, Sierra San Pedro Martir	Baja California	30.892369, -115.481361	2069 m
CIG-1314	pending	23-Apr-18	*Thamnophis hueyi*	Cienega La Grulla, Sierra San Pedro Martir	Baja California	30.892369, -115.481361	2069 m
CIG-1448	pending	19-May-19	*Thamnophis hueyi*	Cienega La Grulla, Sierra San Pedro Martír	Baja California	30.889159, -115.463460	2068 m
CIG-1449	pending	19-May-19	*Thamnophis hueyi*	Cienega La Grulla, Sierra San Pedro Martír	Baja California	30.889159, -115.463460	2068 m
CIG-1494	pending	19-May-19	*Thamnophis hueyi x hammondii*	Cienega La Grulla, Sierra San Pedro Martír	Baja California	30.894320, -115.481086	2066 m
CIG-0810	pending	29-May-16	*Thamnophis* aff. *pulchrilatus*	Near Valle Hermoso, Sierra de Miquihuana	Tamaulipas	22.672603, -99.7941210	2472 m
CIG-0811	pending	29-May-16	*Thamnophis* aff. *pulchrilatus*	Near Valle Hermoso, Sierra de Miquihuana	Tamaulipas	22.672603, -99.7941210	2472 m
CIG-0812	pending	29-May-16	*Thamnophis* aff. *pulchrilatus*	La Marcela	Tamaulipas	23.744825, -99.816433	2490 m
CIG-1848	pending	10-Sep-20	*Thamnophis rossmani*	Municipio de Tepic: between El Armadillo and San Cayetano	Nayarit	21.4392664, -104.842978	924 m
CIG-0500	MZFZ-4593	11-Jul-15	*Thamnophis ahumadai* sp. nov.	Municipio de Talpa de Allende: nr. Cumbre de Guadalupe,	Jalisco	20.169099, -104.711496	2129 m
CIG-1609	MZFZ-4594	23-Aug-19	*Thamnophis ahumadai* sp. nov.	Municipio de Talpa de Allende: nr. Cumbre de Guadalupe,	Jalisco	20.186581, -104.716188	2178 m
CIG-1610	MZFZ-4595	23-Aug-19	*Thamnophis ahumadai* sp. nov.	Municipio de Tomatlán: Cumbre de Guadalupe, Sierra Cacoma	Jalisco	20.157217, -104.675059	2319 m
CIG-1611	INIRENA-2932	23-Aug-19	*Thamnophis ahumadai* sp. nov.	Municipio de Cuautla: Cumbre de Guadalupe, Sierra Cacoma	Jalisco	20.168991, -104.684925	2353 m
CIG-1612	INIRENA-2933	23-Aug-19	*Thamnophis ahumadai* sp. nov.	Municipio de Cuautla: Cumbre de Guadalupe, Sierra Cacoma	Jalisco	20.168991, -104.684925	2353 m
CIG-1613	INIRENA-2934	23-Aug-19	*Thamnophis ahumadai* sp. nov.	Municipio de Cuautla: Cumbre de Guadalupe, Sierra Cacoma	Jalisco	20.168991, -104.684925	2353 m
CIG-1700	INIRENA-2935	21-Jun-20	*Thamnophis ahumadai* sp. nov.	Municipio de Atemajac de Brizuela: Presa near Atemajac	Jalisco	20.1151731, -103.7036033	2422 m
CIG-1701	INIRENA-2936	21-Jun-20	*Thamnophis ahumadai* sp. nov.	Municipio de Atemajac de Brizuela: Presa near Atemajac	Jalisco	20.1151731, -103.7036033	2422 m
CIG-1738	INIRENA 2939	29-Jun-20	*Thamnophis scalaris*	Municipio de Huitzilac: Volcán Chichinautzín	Morelos	19.08675, -99.148971	3278 m
CIG-1781	INIRENA 2937	12-Jul-20	*Thamnophis scalaris*	Municipio de Pinal de Amoles: 1.0 km N of Pinal de Amoles	Querétaro	21.1431, -99.6222	2241 m
CIG-1782	INIRENA 29378	13-Jul-20	*Thamnophis scalaris*	Municipio de Pinal de Amoles: 1.0 km N of Pinal de Amoles	Querétaro	21.1431, -99.6222	2241 m
CIG-1739	pending	29-Jun-20	*Thamnophis scaliger*	Municipio de Atlacomulco: Tecoac	México	19.77559, -99.845494	2525 m
CIG-1740	pending	29-Jun-20	*Thamnophis scaliger*	Municipio de Atlacomulco: Tecoac	México	19.77559, -99.845494	2525 m
CIG-1789	pending	01-Aug-20	*Thamnophis scaliger*	Municipio de Ocampo: 8 km NW of Ocampo, on Hwy. 51	Guanajuato	21.718780, -101.507760	2273 m
CIG-1961	pending	04-Jul-21	*Thamnophis scaliger*	Municipio de Hidalgo: 6.4 km NNE of Mil Cumbres	Michoacán	19.664948, -100.750429	2308 m
CIG-1989	pending	04-Jul-21	*Thamnophis scaliger*	Municipio de Hidalgo: 6.4 km NNE of Mil Cumbres	Michoacán	19.664948, -100.750429	2308 m
CIG-1203	pending	01-Jul-17	*Thamnophis bogerti*	Municipio: San Juan Atepec: Llano de las Flores, Sierra Juarez	Oaxaca	17.440299, -96.508401	2912 m
CIG-1913	pending	19-Jun-21	*Thamnophis validus*	Municipio de Coahuayana: Hwy. 200 at Coahuayana Rd.	Michoacán	18.67476, -103.67911	15 m

Field number	Museum number	Date	Species	Locality	State	Coordinates	Elevation
CIG-1952	pending	05-Jul-21	*Thamnophis vicinus*	Municipio de Charo: pond near Pontezuelas	Michoacán	19.641433, -100.995025	2233 m
CIG-1953	pending	05-Jul-21	*Thamnophis vicinus*	Municipio de Charo: pond near Pontezuelas	Michoacán	19.641433, -100.995025	2233 m
AEVB-0095	pending	03-Nov-17	*Thamnophis chrysocephalus*	Municipio de Los Reyes: Finca Santa Martha	Veracruz	18.652957, -97.009057	1421 m
AEVB-0136	pending	26-Mar-18	*Thamnophis cyrtopsis*	Municipio de Tejupilco: El Tule	México	19.0161, -100.11149	1562 m
AEVB-0005	pending	05-Feb-17	*Thamnophis scalaris*	Municipio de Zacatlán: Valle de piedras encimadas	Puebla	20.024296, -98.050986	2535 m
AEVB-0027	pending	05-Feb-17	*Thamnophis scalaris*	Municipio de Zacatlán: Valle de piedras encimadas	Puebla	20.024296, -98.050986	2535 m
AEVB-0041	pending	19-Nov-16	*Thamnophis scalaris*	Municipio de Acajete: La Joya	Veracruz	19.618235, -97.023109	2170 m
AEVB-0043	pending	19-Nov-16	*Thamnophis scalaris*	Municipio de Acajete: La Joya	Veracruz	19.618235, -97.023109	2170 m
JCSG-0291	pending	28-Apr-19	*Thamnophis conanti*	Municipio de Nogales: Sierra de Agua	Veracruz	18.874395, -97.209868	2249 m
JCSG-0247	pending	21-Sep-18	*Thamnophis sumichrasti*	Municipio de San Juan Tehuacán: El Pedregal	Veracruz	18.618352, -97.047550	2130 m
LOR-0091	pending	28-Jul-18	*Thamnophis chrysocephalus*	Municipio de Zongolica: Tlaquilpa	Veracruz	18.608160, -97.113641	2325 m
LOR-0093	pending	28-Jul-18	*Thamnophis conanti*	Municipio de Soledad Atzompa: Acultzinapa	Veracruz	18.689601, -97.191876	2660 m
RICB-0366	pending	NA	*Thamnophis cyrtopsis*	Municipio de Jacala: La Placita	Hidalgo	20.976902, -99.211789	1425 m
UOGV-3949	pending	15-Aug-20	*Thamnophis conanti*	Municipio de Soledad Atzompa: Acultzinapan	Veracruz	18.689601, -97.191876	2660 m
UOGV-2952	pending	25-Jun-17	*Thamnophis cyrtopsis*	Municipio de Santo Domingo Tonalá: Boquerón de Tonala	Oaxaca	17.63895, -97.94565	1945 m
UOGV-2987	pending	28-Jul-17	*Thamnophis cyrtopsis*	Municipio de Tejupilco: El Tule	México	19.0149, -100.1017	1540 m
UOGV-3932	pending	27-Jun-20	*Thamnophis scalaris*	Municipio de Nogales: Santa Cruz	Veracruz	18.8741683, -97.2063083	2211 m
UOGV-3692	pending	16-Sep-19	*Thamnophis eques*	Municipio de El Tule: Carretera 23 en dirección a Guachochi	Chihuahua	27.0644, -106.26837	1552 m
UOGV-3742	pending	NA	*Thamnophis* aff. *chrysocephalus*	Municipio de Tecpan de Galeana: El Pinito	Guerrero	17.5730, -100.5660	1986 m

Appendix 2

Table A2. Genbank accession numbers used in this study. New sequences generated by us are indicated in bold.

Species	Locality	Catalogue #	Cytb	ND4
Nerodia erythrogaster	USA: Texas, San Saba Co.	CU12550	AF420081	AF420084
Thamnophis ahumadai sp. nov.	Mexico: Jalisco, Atemajac de Brizuela	CIG1700 / INIRENA2935	-	**PP273357**
Thamnophis ahumadai sp. nov.	Mexico: Jalisco, Sierra Cacoma	CIG0500 / MZFC4593	-	**PP273368**
Thamnophis ahumadai sp. nov.	Mexico: Jalisco, Atemajac de Brizuela	CIG1701 / INIRENA2936	**PP273342**	**PP273358**
Thamnophis ahumadai sp. nov.	Mexico: Jalisco, Sierra Cacoma	CIG1609 / MZFZ 4595	**PP273343**	**PP273354**
Thamnophis atratus	USA: California	CU12418	AF420085	AF420088
Thamnophis bogerti	Mexico: Oaxaca	MZFC ART 145	AF420135	AF420138
Thamnophis bogerti	Mexico: Oaxaca, La Doncella	CIG1936	**PP273312**	-
Thamnophis bogerti	Mexico: Oaxaca, La Doncella	CIG1937	**PP273313**	-
Thamnophis bogerti	Mexico: Oaxaca, San Augustin Loxicha	CIG1938	**PP273314**	-
Thamnophis brachystoma	USA: Pennsylvania	CU12379, CAS163984	AF420089	AF420092
Thamnophis butleri	USA: Michigan, Monroe Co.	CU12511	AF420107	AF420095
Thamnophis chrysocephalus	Mexico: Guerrero, Puerto del Gallo	CIG1468	**PP273310**	**PP273352**
Thamnophis chrysocephalus	Mexico: Guerrero, Tecpan de Galeana	UOGV3742	**PP273311**	**PP273375**
Thamnophis chrysocephalus	Mexico: Oaxaca	MZFC-WSB 767	AF420108	AF420098
Thamnophis chrysocephalus	Mexico: Veracruz, Los Reyes	AEVB095	**PP273315**	**PP273350**
Thamnophis chrysocephalus	Mexico: Veracruz, Zongolica	LOR0091	**PP273316**	**PP273370**
Thamnophis chrysocephalus	Mexico: Veracruz, Zongolica	RH13118	**PP273317**	-
Thamnophis "*conanti*"	Mexico: Veracruz, Atzompa	LOR0093	**PP273318**	**PP273371**
Thamnophis "*conanti*"	Mexico: Veracruz, Atzompa	UOGV3949	**PP273319**	-
Thamnophis "*conanti*"	Mexico: Veracruz, Nogales	JCSG291	**PP273320**	-
Thamnophis copei	Mexico: Jalisco, Sierra Quila	CIG1856	**MZ287373**	**MZ287399**
Thamnophis couchii	USA: California, Lassen Co.	CAS165838	AF420103	AF420106
Thamnophis cyrtopsis	Mexico: Hidalgo, Jacala	RICB366	-	**PP273372**
Thamnophis cyrtopsis	Mexico: Mexico State	AEVB136	-	**PP273349**
Thamnophis cyrtopsis	Mexico: Mexico, Tejupilco	UOGV2987	**PP273321**	-
Thamnophis cyrtopsis	Mexico: Michoacan, Tzitzio	CIG1952	**PP273322**	-
Thamnophis cyrtopsis	Mexico: Michoacan, Tzitzio	CIG1953	**PP273345**	-
Thamnophis cyrtopsis	Mexico: Nayarit, Cerro San Juan	CIG1990	**PP273323**	-
Thamnophis cyrtopsis	Mexico: Oaxaca, Santo Domingo Tonala	UOGV3169	-	**PP273373**
Thamnophis cyrtopsis	Mexico: Oaxaca, Boqueron de Tonala	UOGV2952	**PP273324**	-
Thamnophis cyrtopsis	Mexico: Querétaro, Cerro Zamorano	CIG1801	-	**PP273362**
Thamnophis cyrtopsis	Mexico: San Luis Potosí, Sierra de Catorce	CIG1811	-	**PP273363**
Thamnophis cyrtopsis	Mexico: San Luis Potosí, Sierra de Catorce	CIG1812	-	**PP273364**

Species	Locality	Catalogue #	Cytb	ND4
Thamnophis cyrtopsis	Mexico: Sonora, Yecora	CIG2006	**PP273325**	-
Thamnophis cyrtopsis	Mexico: Veracruz, Zongolica	RH13119	**PP273326**	-
Thamnophis cyrtopsis	Mexico: Zacatecas, Villa Gonzalez Ortega	CIG1677	-	**PP273356**
Thamnophis cyrtopsis	Mexico: San Luis Potosí, Real de Catorce	CIG0805	-	**PP273369**
Thamnophis cyrtopsis collaris	Mexico: Guerrero, El Miraval	LACM130112	AF420099	AF420102
Thamnophis cyrtopsis cyrtopsis	USA: Arizona, Pima Co.	ADQ194A	AF420109	AF420112
Thamnophis elegans hueyi	Mexico: Baja California, Sierra San Pedro Martir	CIG1314	**PP273327**	-
Thamnophis elegans terrestris	USA: California, Sonoma Co.	CAS219410	AF420113	AF420116
Thamnophis eques	Mexico: Chihuahua, Guachochi	UOGV3692	-	**PP273374**
Thamnophis eques	Mexico: Puebla, Rancho Las Truchas	CIG1645	-	**PP273355**
Thamnophis eques	USA: Arizona, Yavapai Co.	CU12516	AF420117	AF420120
Thamnophis errans	Mexico: Chihuahua, Guadalupe y Calvo	CIG1862	-	**PP273366**
Thamnophis errans	Mexico: Durango, Charcas	CIG1563	-	**PP273353**
Thamnophis errans	Mexico: Durango, Mil Diez	LSUMZ40836	AF420121	AF420124
Thamnophis exsul	Mexico: Nuevo León, Peña Nevada	CIG1834	-	**PP273365**
Thamnophis exsul	Mexico: Nuevo Leon, Peña Nevada	CAS218283	AF420125	AF420128
Thamnophis foxi	Mexico: Durango, Mil Diez	LSUMZ40846	AF420069	AF420072
Thamnophis fulvus	Mexico: Chiapas, Union de Juarez	CIG1001	-	**PP273351**
Thamnophis fulvus	Guatemala: Quiche	UTA42315	AF420129	AF420132
Thamnophis gigas	USA: California, Colousa Co.	LSUMZ44368	AF420133	AF420134
Thamnophis hammondii	USA: California, San Bernardino Co.	CAS179062	AF420139	AF420142
Thamnophis "lineri"	Mexico: Oaxaca, Sierra Juarez	CIG1203	**PP273329**	-
Thamnophis marcianus	USA: Texas	CU12387	AF420143	AF420146
Thamnophis melanogaster	Mexico: Jalisco, Chapala	CAS165420	AF420147	AF420150
Thamnophis mendax	Mexico: Tamaulipas, Gomes Farías	R. Highton	AF420151	-
Thamnophis nigronuchalis	Mexico: Durango, El Salto	LSUMZ40830	AF420153	AF420156
Thamnophis ordinoides	USA: California, Del Norte Co.	CU12461	AF420157	AF420160
Thamnophis proximus	USA: Texas, San Saba Co.	CU12397	AF420161	AF420164
Thamnophis pulchrilatus	Mexico: Nuevo León, Peña Nevada	CAS 218285	AF420165	AF420168
Thamnophis radix	USA: Colorado, Denver Co.	CAS214183	AF420169	AF420172
Thamnophis rufipunctatus	USA: New Mexico, Catrom Co.	CU12457	AF420173	AF420176
Thamnophis saurita	USA: Florida, Okeechobee	CAS204801	AF420177	AF420180
Thamnophis scalaris	Mexico: Estado de Mexico, Navajas	CAS214292	AF420181	AF420184
Thamnophis scalaris	Mexico: Estado de Mexico, Villa Victoria	LSUMZ42638	AF420185	AF420188
Thamnophis scalaris	Mexico: Morelos, Chichinautzin	CIG1738 / INIERNA2939	**PP273330**	**PP273359**
Thamnophis scalaris	Mexico: Puebla, Zacatlan	AEVB005	**PP273336**	**PP273347**
Thamnophis scalaris	Mexico: Puebla, Zacatlan	AEVB027	**PP273331**	-
Thamnophis scalaris	Mexico: Queretaro, Pinal de Amoles	CIG1781 / INIRENA2937	**PP273332**	-
Thamnophis scalaris	Mexico: Queretaro, Pinal de Amoles	CIG1782 / INIRENA2938	**PP273333**	**PP273360**
Thamnophis scalaris	Mexico: Veracruz, La Joya	AEVB104	-	**PP273348**
Thamnophis scalaris	Mexico: Veracruz, Acajete	AEVB041	**PP273334**	-
Thamnophis scalaris	Mexico: Veracruz, Acajete	AEVB043	**PP273335**	**PP273346**
Thamnophis scalaris	Mexico: Veracruz, Nogales	UOGV3932	**PP273337**	**PP273376**
Thamnophis scaliger	Mexico: Estado de Mexico, Atizapán	CAS214293	AF420189	AF420192
Thamnophis scaliger	Mexico: Guanajuato, Ocampo	CIG1789	**PP273338**	**PP273361**
Thamnophis scaliger	Mexico: Mexico, Tecoac	CIG1739	**PP273339**	-
Thamnophis scaliger	Mexico: Mexico, Tecoac	CIG1740	**PP273340**	-
Thamnophis scaliger	Mexico: Michoacan, Mil Cumbres	CIG1989	**PP273341**	**PP273367**
Thamnophis sirtalis infernalis	USA: California, Santa Clara	LSUMZ37914	AF420193	AF420196
Thamnophis sumichrasti	Mexico: Queretaro	MZFC8869	AF420197	AF420200
Thamnophis sumichrasti	Mexico: Veracruz, San Juan Texhuacan	JCSG247	**PP273344**	-
Tropidoclonion lineatum	USA: Kansas, Geary Co.	CAS174301	AF420205	AF420208

Supplementary material 1

Cytb phylogenetic tree

Author: Jacobo Reyes-Velasco

Data type: pdf

Explanation note: Maximum Likelihood phylogenetic inference of members of the genus *Thamnophis* and closely related genera, based on the mitochondrial gene Cytb.

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Link: https://doi.org/10.3897/herpetozoa.37.e122213.suppl1

Supplementary material 2

ND4 phylogenetic tree

Author: Jacobo Reyes-Velasco

Data type: pdf

Explanation note: Maximum Likelihood phylogenetic inference of members of the genus *Thamnophis* and closely related genera, based on the mitochondrial gene ND4.

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Link: https://doi.org/10.3897/herpetozoa.37.e122213.suppl2

Supplementary material 3

BI phylogenetic tree

Author: Jacobo Reyes-Velasco
Data type: pdf
Explanation note: Concatenated Bayesian Inference phylogenetic tree based on the mitochondrial genes Cytb and ND4 for members of the genus *Thamnophis* and closely related genera.
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Link: https://doi.org/10.3897/herpetozoa.37.e122213.suppl3

Supplementary material 4

Genetic distance

Author: Jacobo Reyes-Velasco
Data type: xlsx
Explanation note: : Genetic distance table of the genetic distances of Cytb and ND4 of numerous samples *Thamnophis* and related Natricinae.
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Supplementary material 5

Large comparable image spread of live photographs of *Thamnophis* related to *Thamnophis. ahumadai* sp. nov.

Author: Christoph I. Grünwald
Data type: jpg
Explanation note: Large spread of high resolution photographs of species related to *Thamnophis ahumadai* sp. nov.
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